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THE BOTANICAL GAZETTE

EDITOR: JOHN M. COULTER

MAY 1923

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EDITED BY

JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

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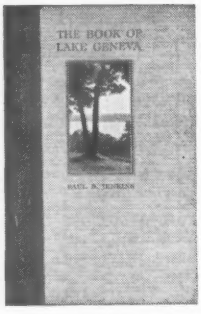

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THE
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INTERMINGLING GAMETOPHYTIC AND SPOROPHYTIC MYCELIUM IN GYMNOSPORANGIUM BERMUDIANUM

H. W. THURSTON, JR.

(WITH PLATES XII, XIII AND FOUR FIGURES)

The genus *Gymnosporangium* comprises a group of rusts interesting historically because of the fact that the first taxonomic description of any rust was a description of a species on *Juniperus*. This genus is also interesting because the aecidial stages of the heteroecious species were for many years supposed to be confined to the family Malaceae, and because of the lack of a repeating or uredospore stage. Recent investigations have somewhat changed our conception of the genus. Members of the Rosaceae, Hydrangeaceae, and Myricaceae have been added as aecial hosts, and in 1916 ARTHUR (1) added *G. nootkatensis*, which possesses true uredospores.

One of the most curious associations of spore forms known among the rusts is that found in *Gymnosporangium bermudianum* (Farlow) Earle on the southern red cedar. This is the only known autoecious species of the genus. It was described by FARLOW in 1887 and has since received only passing mention in the literature. FARLOW (10) first described the aecidial stage on galls from the red cedar in Bermuda. He named the fungus *Aecidium bermudianum*. Not having the teleutospores, he did not recognize the fungus as a *Gymnosporangium*. Galls bearing both aecidia and teleutospores were distributed by SEYMOUR and EARLE in 1872, and they described the

fungus as a *Gymnosporangium*, stating "Teleutospore stage following aecidial stage on the same galls in the spring."

In 1896 UNDERWOOD and EARLE (**26**) noted that "the species, unlike its congeners, produces its aecidial and teleutosporic stages on the same host, from the same gall, and in all probability from the same mycelium." They note also, as did FARLOW, the similarity of the galls to those of *G. globosum*, as well as to another species which has since been described as *G. floriforme*. KERN (**14**) in 1911 described the species somewhat more fully, and gives a photograph.

The fact that teleutosori and aecidia do appear on the same gall, raises the question as to the kind of mycelium from which they arise, and the further question of the relationship of this form to the other gymnosporangia. OLIVE (**19**) has shown evidence for the existence of intermingling sporophytic and gametophytic mycelia in certain autoecious perennial rusts such as *Puccinia obtegens*, on *Cirsium arvense* var. *mite*, *P. Podophylli* on *Podophyllum peltatum*, and *Uromyces Glycyrrhizae* on *Glycyrrhiza lepidota*. It appears that in these cases both kinds of mycelium may be perennial in the same host, each producing the spore form peculiar to it. OLIVE believes such conditions are rather common among autoecious perennial rusts and arose from an assumption by both mycelia of the perennial habit. In *P. Podophylli* he describes the binucleated mycelium as "often invading the immediate neighborhood of the spermogonia, which arise invariably from a gametophytic mycelium." He says further that the aecidiospores are not formed on this gametophytic mycelium, but are secondary in nature and arise from the binucleated mycelium. In *P. obtegens* and *U. Glycyrrhizae* also he has described young uredosori formed of uninucleated cells, with binucleated hyphae pushing up into the sorus and forming spores directly.

Previous to OLIVE's work, SHARP (**22**) had recorded the occurrence of the two kinds of mycelium in the leaves of plants affected with *P. Podophylli*. OLIVE's forms show the sporophytic mycelium crowding out the gametophyte, while in the form I have studied the sporophytic and gametophytic mycelia produce their characteristic spore bearing organs in alternation without interference, although in the same gall.

The gymnosporangia have as yet been studied but little cytologically. BLACKMAN (2), however, gives a full account of the germination of the teleutospore and the accompanying nuclear divisions of *G. clavariaeforme* on *Juniperus communis*, and also of the formation of the spermatia on the leaves of the hawthorn. He did not observe the fusion of the cells at the base of the *Roestelia*, but says it is clear that the transition from the uninucleated to the binucleated condition does take place, as the mycelium at the base of the cup was uninucleated and the spores were binucleated.

STEWART (24), in a paper on the anatomy of gymnosporangium galls, has come to the conclusion that the galls caused by *G. macropus* and *G. globosum* arise from the axils of the leaves, and are transformed axillary buds, but states that both leaf and stem tissue may be involved.

In a later paper and after going over STEWART'S material, WEIMAR (27) reaches the conclusion that the galls caused by *G. Juniperi virginiana* and *G. globosum* originate as modified leaves, and that in no case does more than one abnormally large vascular bundle enter the gall. He also concludes that, in order to reach any considerable size, the galls must be situated close to the stem, and that galls in such a position become attached to the stem and probably involve stem tissue. In this paper is a good summary of the literature dealing with anatomy of *Gymnosporangium*.

Regarding the primitive condition in the rusts, many different hypotheses have appeared in the literature, detailed discussions of which have been given by GROVE (13), OLIVE (18), KERN (15), and others. Concerning the interrelations between autoecism and heteroecism, there have been, broadly speaking, two views. The first, as upheld by OLIVE (18), CHRISTMAN (3), DIETEL (6), KLEBAHN (17), and GROVE (13), is to the effect that primitive rusts were autoecious, and that the heteroecious state arose later by the removal of one spore form to some other host. The other view, as expressed by KERN (15), DIETEL (7), and FISCHER (12), is that primitive rusts were heteroecious, and later became autoecious in many cases by the dropping out of spore forms. In 1898 FISCHER (12) advanced the idea that the primitive rusts were plurivorous, and probably derived from even omnivorous saprophytes. This

idea, although perhaps tacitly accepted by many, has not received the attention it deserves. DIETEL (7) has modified certain of his earlier views, and expressed his belief that ancestral rusts were plurivorous, and that they have gradually become more and more specialized and restricted as to hosts. In this he has partially followed FISCHER, although he still believes that there are two ways in which heteroecism may have arisen, that is, from other forms already heteroecious (as he believes the whole family Melampsoraceae to have done), or in some instances from autoecious forms, as in the case of *U. Veratri*, which bears its aecidia on *Adenostyles* spp., and which he would derive from *U. Cacaliae*, an autoecious form on *Adenostyles*. DIETEL thinks that *Gymnosporangium Elissii* is a case in which a spore form has jumped to a new host, in this case *Myrica* outside the family Malaceae to which most of the Gymnosporangia are restricted, implying that the parasite regains in some measure its original plurivorous habit. It is of course possible to regard the life history of *G. Elissii* as a good example of the maintenance of an earlier more plurivorous condition which doubtless prevailed in the genus. DIETEL (6) expressed the opinion that *G. bermudianum* is a primitive form which has not yet become restricted to alternate hosts. Later (7) he considers it to be analogous to the autoecious *Melampsora amygdaloidis* on *Salix*, and that both of these species have arisen from heteroecious forms by the removal of the aecidium to the teleutospore host. He has given no indication, however, as to how he would fit either of these rather unusual forms into the larger idea of a plurivorous ancestral condition.

For the sake of making possible the utilization of all possible morphological characters in determining the phylogenetic relations of *Gymnosporangium*, it is worth while to note the differences between *Roestelia* and *Aecidium* according to the original descriptions. REBENTISCH'S diagnosis of *Roestelia* is as follows:

Roestelia Reb. Prod. Florae Neomarchicae Ber. 1804, p. 350.—Peridium filis parallelis apice cohaerentibus compositum e stromate progrediens. Pulvis farinaceus nudus. He makes *R. cancellata* the type and adds the further description:

Aestate in foliis ut macula dilute crocea et punctata apparet, dein tubercula spadicea ibidem proveniunt quae tandem ut peridia conica prominent. Pers.

Ob peridii structuram proprium genus constituere meretur; itaque ab Aecidiis disjunxi et ex aestumatiss. amici mei Roestel (Pharmacopoli Landsbergensis) cui haec florula multum debet, nomine Roesteliam vocavi.

PERSOON diagnoses *Aecidium* as follows:

Aecidium Pers Syst. Nat. 2:1472. 1791.—Theca (membranacea) utrinque glabra, feminibus nudis non cohaerentibus plena.

REBENTISCH (Prod. Florae Neomarchicae Ber. 1804, p. 351) diagnoses *Aecidium* as follows:

Peridia manifesta teretia, membranacea, demum nupta ore plerumque dentato. Pulvis farinaceus coloratus nudus.

KERN (14) finds it possible to make use of the degree and kind of cohesion of the apical cells of the peridium and the method of its dehiscence in keying out the species of *Gymnosporangium*. Further study of young and vigorously growing material may make it possible to utilize these characters for phylogenetic groupings within the genus.

Material and methods

Material for cytological investigation was accumulated from all available sources. An endeavor was made to secure viable spores for cultures, to determine if possible which spore form was capable of infecting the cedar. I have had material of *G. bermudianum* from Bermuda, Florida, and Mississippi. The greater part of the material, however, was collected near Daytona, Florida, by Dr. R. A. HARPER in 1916, and by Dr. B. H. DOUGLAS in 1917, and I am also indebted to Dr. WORTLEY and Professor H. H. WHETZEL for specimens from the Bermuda Islands, the type locality.

G. bermudianum from Florida and Mississippi has for its host *Juniperus barbadense* and *J. virginiana* L., while the specimens from Bermuda were on *J. bermudianum* L. *G. bermudianum* is also reported on *J. Lucayana* Britt. from the Bahamas.

Both Dr. HARPER and Dr. DOUGLAS while collecting in Florida found some galls very similar to those of *G. bermudianum*, but considerably larger. Some of these large forms were "smooth," and looked very much like *G. macropus*, except for a very slightly scaly appearance. Others were quite warted, lobed, or tuberculate, and agreed more nearly with the descriptions of *G. floriforme*. On

none of these larger galls were aecidia to be found, although the scaly surface of the ones which were not lobed or warted suggested that an aecidial layer might perhaps have been present earlier in the season. Material has also been secured from Texas which agrees in character with these large galls from Florida, and suggests a possible transition from *G. macropus* or *G. globosum* to *G. floriforme* or even to *G. bermudianum*.

At Biloxi, Mississippi, according to Dr. TRACY, the host is quite abundant, but the fungus is restricted to a small percentage of the trees. In Bermuda, in the vicinity of Paget East, the fungus is somewhat more abundant, occurring on 25–40 per cent of the red cedars, according to WORTLEY.

Specimens of *G. bermudianum* collected at Biloxi, Mississippi, in January and February, 1916, and in Florida during January and February, 1917, did not show teleutospores at all, while the old aecidium cups, often still containing spores, were to be found on nearly every gall. Out of 150 galls only 3 did not show aecidia. Other specimens from Biloxi, collected from March 9 to May 12, 1916, bore teleutospores very sparingly, only 10 galls out of a lot of 241 showing them. Out of this same lot there were 42 galls which appeared perfectly smooth. Many of these, however, were proved to contain young aecidia when subsequently sectioned. Material from Biloxi collected during October and November showed the aecidia in their prime. The galls collected during the period March 9 to May 12, 10 of which bore teleutospores, were all kept under observation in the greenhouse for a period of two weeks or more, and an attempt was made to force more of the teleutosori by placing the twigs bearing the galls in small bottles of water and covering with a belljar, to keep them moist. Three more of the galls did produce teleutosori under this treatment, but the sori were poorly developed. It is probable, however, that under natural conditions a far larger percentage of the galls would have produced teleutospores. Spermagonia have never been observed in connection with *G. bermudianum*, and are assumed to be lacking.

For cytological investigation, material from the foregoing collections was fixed in Flemming's weaker solution and in Bouin's solution. The Bouin solution gave the most reliable and uniform

results. The usual method of washing and imbedding in paraffin was followed, with the exception that the material was often allowed to remain in the paraffin oven as long as 3–4 weeks. This was necessary especially after the Flemming fixation in order that perfect infiltration might take place. Sections were stained with the Flemming triple stain and also with fuchsin and iodine green, which gave very satisfactory differentiation of the mycelium as well as the host tissue.

Spore germination

Aecidiospores from some of the material collected in the fall of 1916 were tested for germination. Spores were floated on tap water in Syracuse watch glasses, and also dusted over the surface of agar plates. Germination took place readily in the watch glasses and was complete in 2–4 hours. The long germ tubes (fig. 1) are typical of aecidiospores, and they contain two nuclei which lie close together and usually near the middle or tip end of the tube. Aecidiospores from material collected in the winter and in the spring did not germinate.

Teleutospores from material collected in the spring of 1916 were germinated in the infection flames by spraying them with water and then allowing germination to take place in moist air. It was observed that only a small percentage of the spores in any sorus germinated. The basal cell of the spore usually germinated first. The spores in sori produced in the greenhouse as a result of forcing, as previously described, did not germinate at all. A few 3-celled teleutospores were observed and also a few 1-celled spores. When teleutospores were floated on water in watch glasses in the same method used for aecidiospores, the germ tubes, instead of becoming promycelia, simply grew very long. This was probably the result of being too moist or of becoming submerged.

Cultures

In an attempt to determine whether aecidiospores or basidiospores or both were capable of infecting the cedar, several cultures were made as follows. Young seedlings of *Juniperus barbadense* were raised in the greenhouse for this purpose. Trees 6–18 inches

tall and in a vigorous growing condition were selected and placed in a moist infection frame. Suspensions of spores were sprayed upon the trees with an atomizer. Masses of spores also were rubbed upon branches and leaves, and galls producing spores were hung over them. The trees were allowed to remain 48 hours in the infection frame, and were then set out upon the greenhouse bench. It was ascertained by germination tests at the time of inoculation that the spores used were in viable condition. The results are shown in table I. The trees were kept under observation for nearly two years, but in no case were infections found.

TABLE I

Date of inoculation	No. of trees	Kind of spores (from Biloxi, Miss.)	Result
5–12–16	8	Teleutospores	Negative
10–29–16	12	Aecidiospores	Negative
11–9–16	12	Aecidiospores	Negative

An attempt was also made to transfer the fungus to some alternate host. In this experiment aecidiospores only were used. Young potted plants of apple, *Crataegus*, and *Amelanchier* were sprayed with spore suspensions on the same dates as the preceding, and kept in a moist infection frame for 48 hours. In this case also the results were all negative.

Structure of galls

WEIMER (**27**) came to the conclusion that galls caused by the species of *Gymnosporangium* which he studied (*G. Juniperi-virginiani* and *G. globosum*) always originated as leaf infections, but that especially in older galls or perennial forms stem tissue may become involved. He mentions the fact that the terminal portion of the leaf remains attached to the gall and may be found there for some time. His contention that STEWART has mistaken a normal axillary bud for a young gall seems to the writer hardly to be justified. That the galls of *G. bermudianum* also originate from infection of the axillary bud seems probable from the evidence at hand, although there is no doubt that most of them involve both leaf and stem tissue. Figs. 7 and 8 show a young gall which in this stage

seems to be nothing else than a hypertrophied axillary bud. Figs. 9 and 10 are older and show the leaf pushed up and still attached to the gall, as described by WEIMER. In these older galls, however, at least part of the basal portion of the leaf which subtended the earlier stage has also gone to form gall tissue. It seems probable from the splitting of the leaf as shown in these figures that more or less of its tissue has simply shrivelled up.

The galls produced by *G. bermudianum* are small, usually globular in shape, and rarely over a quarter of an inch in diameter. They are often clustered three or four close together and usually close to the stem. The color is reddish to purplish brown. The surface is smooth and lustrous, becoming somewhat scaly and roughened in old specimens. Dried specimens become quite shrunken and wrinkled, often leaving the aecidium cups protruding as small papillae. The color of the dried specimens does not change. The surface layer of the galls is corky. This cork layer is developed when the gall is very young. Inside the cork layer the gall tissue is very largely made up of large parenchymatous cells. Vascular elements are to be seen in nearly every section. The vascular bundles appear much branched and proliferated, the strands penetrating the gall in all directions, gradually breaking up and connecting with the parenchymatous cells. The mycelium of the fungus is very abundant and appears everywhere, ramifying through the gall between the cells.

Proliferation

Some of the galls show a peculiar secondary growth or proliferation of the gall tissue itself. This new growth appears about the base of an old and apparently dead gall, and gives somewhat the appearance of a twin gall. Such a condition is pictured in fig. 11, and was noticed in about one-third of over one hundred specimens from Bermuda. It seems probable that the mycelium has penetrated the stem and remained dormant there, while dying out in the gall proper, until conditions again become favorable for growth, when new gall formation takes place. Only binucleated mycelium has been found in these proliferations, which would give rise to teleutospores. Sori, however, have not been observed on these

proliferations. It is suggested that the proliferation may be the means by which the fungus is kept alive during unfavorable periods for growth, and that the sporophytic mycelium is essentially perennial, but only lives over in the deeper portions of the tissue. DODGE (**8**) has found in the case of *G. Elissii* (a stem attacking form) that the mycelium "invades every tissue except cork."

Roestelia

The roesteliae appear scattered upon the galls (fig. 12), which may bear from one to a dozen or more, according to the size of the gall. They are deeply imbedded in the gall tissue, and surrounded within the gall by a pseudoparenchyma of fungus hyphae. There is always an abundance of fungus tissue about the bottom and sides of the aecidium. The peridium forms a narrow tube extending some distance above the surface of the gall and spreading slightly; it is fragile and soon broken up. The roestelia appears to start when the gall is quite young and to grow up with the gall, at least such would be the inference from its position in the tissues. Some aecidia are never able to rupture the surface tissues, but still appear to be full of entirely normal spores. Mature aecidia have been found still imbedded 4–5 cells beneath the epidermis. The material shows that the aecidia always arise from a uninucleated mycelium (text fig. 1).

It has not been my good fortune to be able to examine aecidial material in the very young stages, but I have nevertheless been able to find fairly abundant evidence that the binucleate condition arises by the fusion of two gametophoric hyphae in the base of the aecidium, as described by CHRISTMAN (**3**), FROMME (**11**), and others for the more open types of aecidium cups. Fig. 4 shows such a fusion cell. Multinucleated cells have been described (OLIVE **19**, RICHARDS **21**, FROMME **11**) at the base of various types of aecidia. Occasionally such cells are to be found in my sections of *G. bermudianum*. Fig. 5 shows a 4-nucleated cell, probably the result of the fusion of four cells, although in this case the fusions have not given rise to a spore chain.

KERN states that the aecidia of *G. bermudianum* arise from a biennial mycelium, but it seems from my studies that the game-

tophytic mycelium is shorter lived than the sporophytic, which can be demonstrated in the galls at almost any time of year. Galls sectioned in the winter after the aecidia have ceased to bear spores show a peculiar appearance of the mycelium, which for a considerable distance about the cup appears as if the hyphae were all dead. This mycelium takes the stain intensely, and no differentiated structures or nuclei are visible. Somewhat deeper in the tissue mycelium

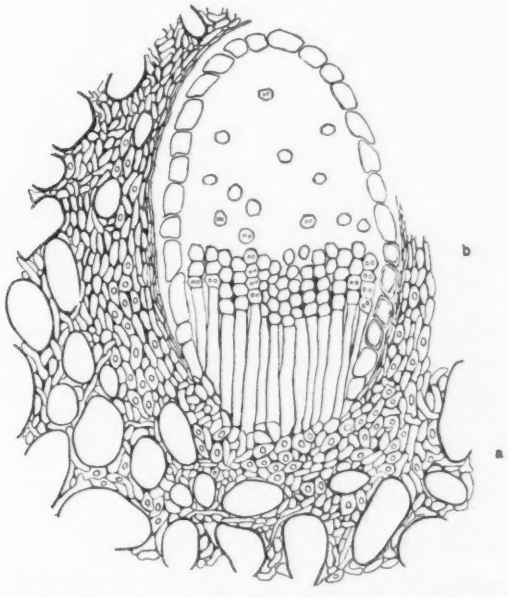

FIG. 1.—Section through mature aecidium, showing *a*, uninucleated mycelium at base, *b*, binucleated spores; somewhat diagrammatic.

may be found which stains normally and shows two nuclei to the cell. This mycelium would presumably give rise to teleutosori. The gametophytic mycelium is not in any sense crowded out by the sporophytic mycelium. Both kinds appear to function normally, although the gametophytic mycelium, so far as I have observed, does not persist after fruiting.

The basal cells from which the chains of spores arise are very long, which might be expected when the long and tubular roesteliae

are compared with the broader, more open, and cup-shaped aecidia. The length of these cells is from one-fourth to one-third the length of the cup itself. They appear simply as parallel hyphae and are sometimes septate.

Teleutosorus

The teleutosori are born apparently anywhere on the surface of the gall. Usually only a few (1–4) on a single gall, they appear as low, rounded masses, dark cinnamon brown in color. A teleutosorus may be found immediately beside an aecidium cup. The sections have shown (fig. 6) that the teleutosorus arises from a binucleated mycelium which can usually be traced throughout the

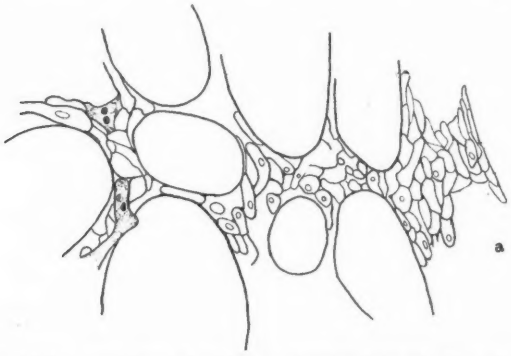

FIG. 2.—Section near base of aecidium, showing intermingling of sporophytic and gametophytic mycelium: *a*, parenchymatous mycelial tissue surrounding aecidium; drawn with camera lucida and 1/12 oil immersion lens.

extent of the gall. At first an aggregation of hyphae appears under the epidermis, which is pushed up, the hyphal tips appearing in quite an orderly palisade. Buffer cells, as described by DODGE (**8**, **9**) for several other species of *Gymnosporangium*, are present, although I am not able to say that only the ultimate cell serves in this capacity, or that the penultimate cell always becomes the basal cell. In older sori it would appear that teleutospores may arise from cells which are situated 2 or 3 cells removed from the ultimate cell. From a basal cell a cell is cut off, which elongates, and finally divides to form the spore and its stalk. Several stalks may arise from a single basal cell, much as described by CHRISTMAN (**4**). The sporophytic hyphae have a noticeably greater diameter than

the gametophytic hyphae, and, as already mentioned, the binucleated cells of the sporophytic mycelium can be demonstrated in the galls, even when no teleutospores are being produced. Hyphae with binucleated cells have even been observed close around the pseudoparenchyma of a vigorous aecidium cup (text figs. 2, 3). In cases of this kind, however, it is not always possible to tell with certainty whether such cells belong to a sporophytic mycelium, or whether they may not possibly be gametophytic cells in which the septum has not been laid down, following a nuclear division. From the size and staining reactions the writer believes this to be true sporophytic mycelium. From the prevalence of binucleated mycelium in so many galls which are bearing aecidospores, and from the "dead" appearance of the gametophytic mycelium after its spores have been produced, it would appear that the sporophytic mycelium is longer lived than the gametophytic, perhaps in some cases perennial (text fig. 4).

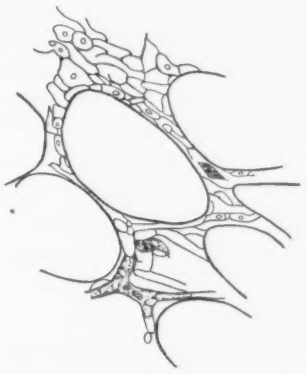

FIG. 3.—Section near base of aecidium, similar to fig. 2, showing intermingling of sporophytic and gametophytic mycelium; drawn with camera lucida and 1/12 oil immersion lens.

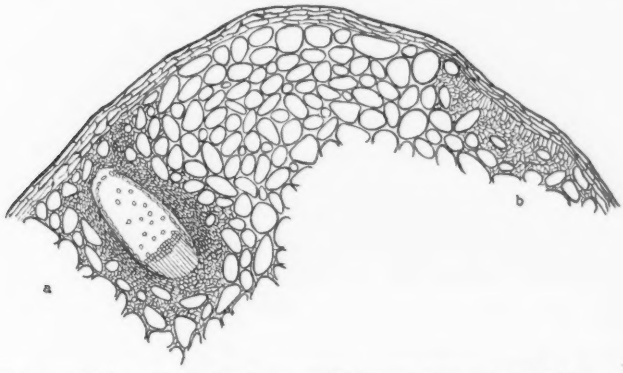

FIG. 4.—Section of gall, showing *a*, aecidial sorus from uninucleated mycelium, *b*, teleutosorus from binucleated mycelium; slightly diagrammatic.

Many of the galls bear scars in the form of a hollow or sunken area, sometimes blackened, but at any rate lacking the typical cork epidermal layers. From their position, number, and general appearance I have interpreted these as the scars of teleutosori of previous years. If this is a correct interpretation, it is in support of the view we have here of a perennial or biennial sporophytic mycelium.

Cell invasion and haustoria

Haustoria are very numerous, and the material appeared to be favorable for a study of the old question as to whether or not the haustorium really enters the cell, that is, penetrates the plasma membrane, or merely invaginates it. COLLEY (**5**) found the white pine blister rust to be favorable material for the study of haustoria. He states that haustoria in *Pinus Strobus* are always uninucleated (which is perhaps to be expected, since the mycelium is uninucleated), and mentions a sheath, which he says resembles the cell wall of the host, but which appears only in old or mature haustoria. He also states that the plasma membrane is not "broken or pierced by the haustorium."

OLIVE (**20**) states that the haustorium of *Botryorhiza Hippocrateae* pushes in the plasma membrane of the host cell which it invades. The haustoria of this form are multinucleated, and form masses which may almost fill the host cell. DODGE (**8**) reports large-sized binucleated haustoria in *G. clavipes* and *G. Elissii*.

SMITH (**23**) undertook a really critical study of haustoria. He describes a thickening of the cellulose wall of the host cell opposite the point of penetration of the fungus, which he believes is due to the deposition of additional cellulose by the plasma membrane stimulated at this point by the attempts of the fungus to enter the cell of the host. Further, he describes the mature haustoria as surrounded by a sheath, and the sheath as being bounded by the plasma membrane. The contents of the sheath he believes to be the disintegrating cellulose of the ingrowth of the wall. It is SMITH'S contention that the haustorium does not penetrate the plasma membrane (at least in the early stages), as is shown by the ingrowth of the cellulose of the host cell wall.

The haustoria of *G. bermudianum* (as well as those found in sections of some of the larger galls previously mentioned) both from gametophytic and sporophytic mycelium contain regularly one nucleus. No exceptions to this rule have been observed. There is what I regard as an appressorium, formed on the outside of the cell wall where a haustorium is to be formed (figs. 2, 3). It appears as a thick-walled, rounded-up segment of the hypha, closely pressed against the wall of the host cell, and cut off from the rest of the mycelium by a septum. This organ, which I have called an appressorium, appears to be comparable with the organs in the mildews and certain of the Perisporiaceae, which serve as attachment organs and give rise to haustoria. It is also similar to organs described by LUTMAN (**17a**) for certain smuts. Its function would here be a similar one, serving to hold the fungus mycelium closely appressed to the host cell until penetration is accomplished. From the appressorium a slender hyphal branch grows into the host cell wall to form the haustorium. At first there is a thickening of the host cell wall opposite the point of attack. Whether this is due to gelatinous swelling of the wall, or whether new cellulose is deposited is not clear. In either case the young haustorium soon catches up and penetrates the wall. The plasma membrane (fig. 2) of the host cell is merely thickened about this cellulose papilla or ingrowth, and is very granular in appearance. In later stages the cellulose papilla disappears, and there is often an actual depression in the host cell wall where the haustorium passes through. The haustorium often has a sheath or membrane about it which is wrinkled or shrunken away from the haustorium, and in such cases can clearly be seen. The fact that there is such a noticeable sheath would seem to prove that the plasma membrane is not yet ruptured. A mere invagination of the membrane, however, might be expected to draw the membrane away from the cell walls for a considerable distance on either side of the point of penetration. Such a phenomenon has not been observed, and the membrane appears closely appressed to the cell wall right up to the penetrating hypha, and often follows the contour of the haustorium itself so closely as to be scarcely visible. The haustoria are especially numerous in the vicinity of the vascular elements.

That portion of the parasite which actually penetrates the cell wall of the host is amazingly fine and threadlike. Although the intracellular portion may become greater in diameter than the normal mycelial hypha, the nucleus must pass through the cell wall through this slender threadlike tube. Whatever nourishment the parasite gets apparently must also pass through these tubes in a manner similar to the translocation of plastic or dissolved food materials through the so-called protoplasmic connections which exist between the cells of many plant tissues. It is a noticeable fact that the middle lamellae of the host cell walls are rarely visible when the fungus is active, and the walls themselves are much swollen and appear almost hyaline, taking the stain very faintly.

Discussion

DIETEL (7), in his most recent discussion of the heteroecious rusts, has accepted in part the hypothesis of FISCHER, and believes that the ancestral rust was plurivorous after the manner of saprophytic fungi, and in contrast with the very pronounced specificity in choice of host plants that many of the rusts exhibit today. He believes, however, that there are two ways in which heteroecism may have arisen: (1) from species already heteroecious by the removal of one spore form to another host (here he cites as an example *G. Elissii* on *Myrica*, which he thinks of as having arisen from some other species of *Gymnosporangium* with aecidia on Malaceae); (2) from autoecious species, giving here as an example *U. Veratri* with aecidia on *Adenostyles*, which he would derive from *U. Cacaliae* which is an autoecious form also on *Adenostyles*. The latter method (7) he now admits is much in the minority, although he earlier (6) believed that all heteroecious species originated in this way. He further believes that some of these changes are now going on, the spore form that makes the change to a new host reassuming in a manner its plurivorous habit, while at the same time certain other species are changing from heteroecism to autoecism, becoming more specialized by the dropping out of spore forms. Such "reduced species" have appeared as a rule upon the aecidial host of the heteroecious species, and may or may not have resulted in the total disappearance of the heteroecious form. According

to DIETEL, *Chrysomyxa Picea* on *Abies* in India is a good case of a reduced form that has arisen from a heteroecious one. *Melampsora Amygdaloidis* (*Amygdalinae* Kleb.), an autoecious species with the full complement of spore forms on *Salix amygdalina* and *S. pentandra*, he also considers as having arisen from a heteroecious species (perhaps like *M. Larici-pentandris* with *Caeoma* on *Larix*), but in this case on the teleutospore hosts of the original species.

It seems apparent that FISCHER and DIETEL have offered a stimulating suggestion in the idea that primitive rusts were plurivorous. DIETEL in particular has not, in his own mind, accepted all that this implies. A primitive plurivorous condition fits in well with ideas of the development of parasites from saprophytes as pointed out by FISCHER (**12**), and lately well expressed by THOMAS (**25**). Beginning with plurivory, and becoming more and more restricted and dependent as the parasitic habit has become fixed, certain of the rusts have come to live on two sets of hosts, while others, as for example certain biological strains or races of *P. graminis*, have become further specialized and limited almost to a single species for each generation. Still others, the autoecious forms, have become restricted to a single species or limited group of hosts. Among these forms we find all degrees of restriction, from forms like *P. malvacearum*, which will attack practically every genus in the mallow family, to forms like *P. Buxi*, which is, so far as known, confined to the single species *Buxus sempervirens*. It is even possible that certain forms on perennial hosts such as the thistle rust are becoming restricted to individuals of the species, and may even be losing their power to infect the host by means of their spores. Such restriction and increasing specificity have not been all in one orderly line of course, and may not serve to explain all the differences and relationships between heteroecious and autoecious species. It is not to be disputed that there is a possibility in certain cases of development in the opposite direction, namely, from the more specialized to the less. It is believed, however, that the idea of greater and greater specialization and restriction pictures quite accurately the general tendencies of the group. On this basis it would appear logical to explain the species which DIETEL has considered as arising from autoecious forms

by the fact that they have longer retained their primitive plurivorous habit, rather than by assuming (as he apparently does) that they have become specialized and restricted, and then in some mysterious manner regained the power to infect a new type of host.

It seems apparent, therefore, that while exhibiting this tendency to become more and more restricted, the rusts as a group are still in a plastic state, and that consequently we may expect to find all possible modifications of the usual life history. It is suggested that tropical and semitropical forms of rusts may well be in a more plastic state as regards their host relationships than are the rusts of more temperate climates. Evidence is accumulating that infection occurs only on actively growing regions of the host. Where conditions are such as to keep the host in a continuous growing state, new infections may occur repeatedly in a single year. The rusts of the colder climates have of necessity had to adjust themselves to the seasonal changes of their hosts, and have thus probably gained a much greater fixity in their developmental cycles.

G. bermudianum may be considered as a reduced specialized species which has learned to live on a single host, without however having lost any spore form except spermatia. It is not impossible that these may yet be found. If we assume with OLIVE (**18**) and others that the ancestral rusts were autoecious upon their present gametophytic hosts, we should have to assume that *G. bermudianum* has descended from a totally different ancestor from any other species of the genus, since it is the only one with a gametophytic stage on a juniper. This argument, however, is not very convincing. If the developmental tendency has actually been from autoecism to heteroecism (OLIVE **18**, DIETEL **6**, GROVE **13**), then we are bound to consider *G. bermudianum*, because of its autoecism, as the most primitive of the Gymnosporangia, while at the same time it possesses a highly developed and specialized type of aecidium. On the other hand, if plurivory has been the ancestral primitive state, we might expect to find some of the Gymnosporangia becoming autoecious on one host or the other. DIETEL (**6**) expressed his opinion that this form is a primitive one, not yet restricted to alternate hosts. He has since (**7**) stated that he considers it to have

originated from a heteroecious form. By accepting the hypothesis of plurivory as the ancestral condition, it is not necessary to imagine any fixed intermediate state in which this form was restricted to alternate hosts, but it is by no means impossible that *G. bermudianum* may have a roestelial stage on some species of *Crataegus* in the south. The discovery of such a form would be most interesting in its bearing on the whole problem of heteroecism. As it is known now, it is the most advanced and specialized species of the genus. Because of its semitropical habitat it seems possible that this rust may be in a plastic state. It is not impossible that under suitable conditions of artificial inoculation one might be able to infect with it some species of *Crataegus* or other malaceous host.

Relationships

Reasoning from host relationships alone, an attempt might be made to show that some sort of relationship exists between *G. bermudianum* and the species of *Peridermium* which also have aecidia on conifers. Morphologically, however, the roestelia of *G. bermudianum* are of course more similar to the aecidia of the *Puccinia* group of rusts than to the aecidia of *Peridermium*, although these also show relationships, particularly through the leaf inhabiting forms, to the *Puccinia* type of aecidium. *Roestelia* was first described as a genus in which the peridial cells remained coherent at the tip of the peridium. Within the genus *Gymnosporangium* there is a nice transition from this type, as found in forms like *G. juvenescens*, to the more open and cuplike aecidium which in forms like *G. Elissii* on *Myrica* show all the characters of the aecidium as originally described. This fact was pointed out by Kern (14), and undoubtedly shows, as he has suggested, in what species *Gymnosporangium* is most closely related to the *Puccinia* group of rusts. *G. bermudianum* itself, while its peridium dehisces early, is deep seated within the host, and is so markedly tubular in character that it may be said to conform more nearly to the roestelial type than to the open and cupulate aecidial type, which fact, however, affords no evidence as to the effect of the host on the morphology of this spore form. This fruit form is certainly as true a roestelia as many of those on malaceous hosts.

INTERMINGLING MYCELIA

In the case of the perennial forms described by OLIVE (19), it has not been shown how infection may take place, nor how the binucleate condition arises when the gametophytic mycelium produces only spermogonia. Presumably in these three forms, *P. Podophylli*, *P. obtegens*, and *U. Glycyrrhizae*, both the sporidia and the secondary aecidiospores and uredospores may under suitable conditions infect the host. No one has shown in the case of an autoecious or perennial rust how close the spatial association may be between spores produced on the one hand from gametophytic and on the other hand from sporophytic mycelium. There are many cases known, of course, where uredospores and teleutospores are produced in the same sorus, one being in *Gymnosporangium* (*G. nootkatensis* on *Chamaecyparis nootkatensis*). OLIVE has described young sori formed of uninucleated mycelium in which, however, the spores are formed by binucleated hyphae which push in and suppress the uninucleated mycelium. In many autoecious rusts the two kinds of mycelia apparently produce their respective spore forms in close proximity and in succession on the same leaf. *U. Cunninghamianus* was described by BARCLAY (1a) as producing teleutospores within the aecidium cup, each sorus presumably arising from a separate infection by the other spore form. The genera *Allodus* and *Pucciniola* as described in the *North American Flora* present numerous instances of a similar association of spore forms. In *G. Bermudianum* the two kinds of mycelium exist side by side in a very close relationship within a single gall, and each produces its spore form apparently unhampered in any way by the presence of the other. As DIETEL notes, *Melampsora amygdaloidis* on *Salix* is a form analogous to *G. bermudianum* in being autoecious upon the host which commonly bears the teleuto generation of other species of the genus. This form has been cultured by KLEBAHN (16), who has succeeded in bringing about infections with both sporidia and aecidiospores. It does not form a gall, and while it might well be an interesting subject for cytological investigation, it cannot be presumed to differ from many other autoecious species. Since cultures of *G. bermudianum* have so far been unsuccessful, we can only speculate on the probabilities as to the infection phenomena.

The problem as to how the two spore forms from different mycelia come to be found in one and the same gall is quite different from that presented by perennial forms such as *P. Podophylli*, *P. obtegens*, and *U. Glycyrrhizae*, with which OLIVE worked. In these forms both kinds of mycelium exist throughout the host from year to year, and in the case of the thistle rust winter in the running rootstocks, and presumably in the underground parts in the other two forms, while the galls of *G. bermudianum* are formed anew every year or two years, and yet they bear two spore forms from two kinds of mycelium.

The problem here presented is also believed to be different from that presented by the *Allodus* and *Pucciniola* forms, for while these forms where the teleutosori arise within the aecidium cup need cytological investigation, it seems probable that we are not here concerned with intermingling mycelium, but that the so-called secondary aecidia which are really the morphological equivalent of uredosori arise from the same binucleated mycelium which later bears the teleutospore. If we assume that the aecidiospores only can infect the cedar, we should expect galls to be formed which would bear teleutospores first. How then can we account for the presence of the uninucleated mycelium which forms the aecidia? On the other hand, if we assume that sporidia from teleutospores infect the cedar, then we should expect the aecidium to be, as it probably is, the first sorus form produced, but we should still be at a loss to account for the appearance of a binucleated mycelium bearing teleutospores on the same gall.

It would perhaps be more natural to imagine that the cedar is first infected by aecidiospores, as is the case in all other Gymnosporangia, and that the gall itself is then reinfected by the sporidia from germinating teleutospores. In my sections I have examined few galls that I can say with certainty have contained only one kind of mycelium. Aecidia are without doubt found on very small galls, so small that there is room for only one aecidium. Such of these galls as I have sectioned show only uninucleated mycelium, although in larger galls it is impossible to say with assurance that only one kind of mycelium is present; in fact, galls that only show externally one of the spore forms have almost uniformly revealed in sections the presence of both kinds of mycelium.

There is some evidence that both teleutospores and aecidiospores can infect the cedar, as has been shown in the case of *Melampsora amygdaloidis*, where both spore forms of this rust are capable of infecting its host, *Salix*. It seems quite probable that in reality there are three kinds of galls, some bearing teleutospores, some aecidia, and some both, and it would be entirely possible for aecdiospores to germinate while still in the cup and to reinfect the gall.

From the evidence that in proliferating galls only sporophytic mycelium has been found, which is probably perennial in character, it would seem that we have here all the possibilities of a case parallel to the early spring production of teleutospores in the *Podophyllum* rust, and further ground for the assumption that there are three kinds of galls. The sporophytic mycelium in the proliferations could give rise to teleutospores, which upon germination may be supposed to reinfect the cedar and give rise to galls bearing at first only aecidia; some of these by reinfection of the same gall giving rise in turn to a second crop of teleutospores, this time upon the aecidium bearing galls.

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EXPLANATION OF PLATES XII, XIII

PLATE XII

FIG. 1.—Germinating aecidiospore, showing two nuclei in germ tube.

FIG. 2.—Very young stage in formation of haustorium, showing appressorium containing two nuclei.

FIG. 3.—Mature haustorium with one nucleus, showing also appressorium and binucleated intercellular mycelium.

FIG. 4.—Fusion cell from base of aecidium, showing origin of binucleated condition.

FIG. 5.—Four-nucleated cell from base of aecidium.

FIG. 6.—Young stage in formation of teleutosorus, showing binucleated mycelium.

PLATE XIII

FIGS. 7–10 taken with Carl Zeiss micro planar 35 mm. focal length, all ×6; figs. 11 and 12, × 1.5.

FIG. 7.—Very young gall of *G. bermudianum*, showing its origin in axil of leaf.

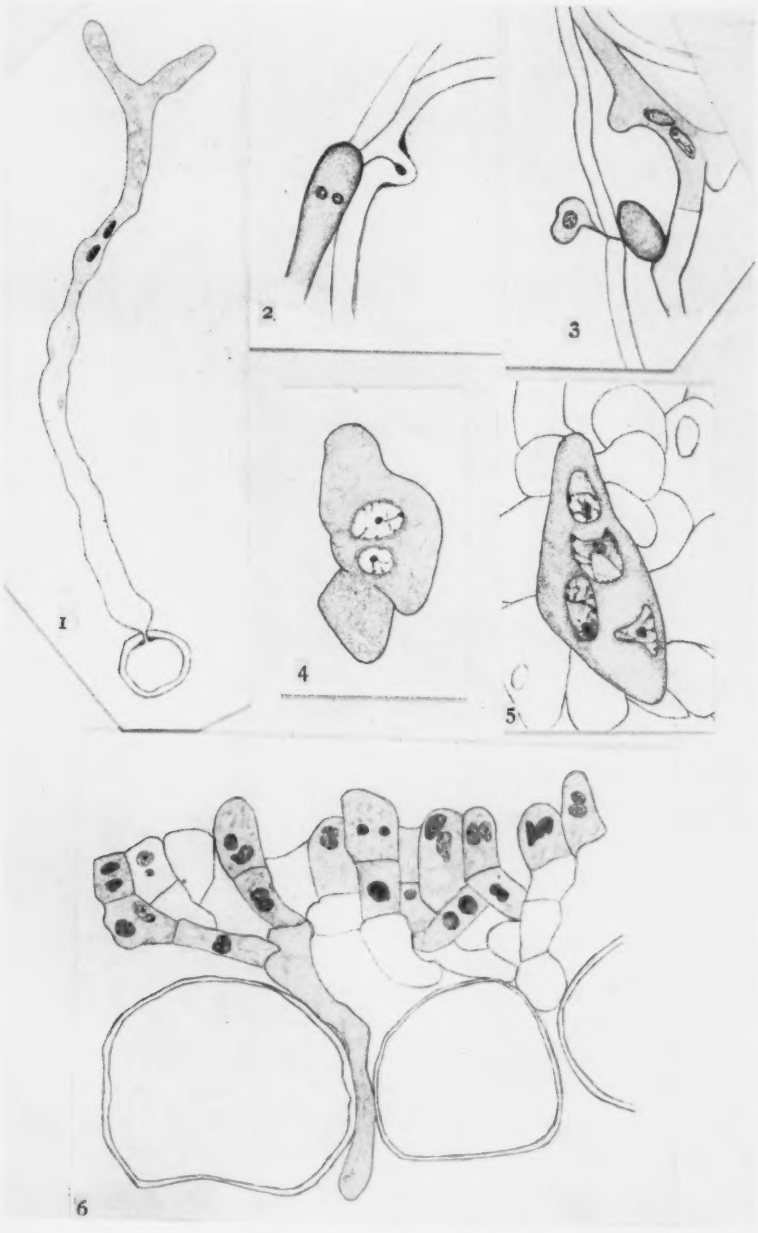
FIG. 8.—Same gall with most of surrounding leaves dissected away.

FIG. 9.—Larger and older gall, showing persistence of leaf, no sori as yet appearing on this gall.

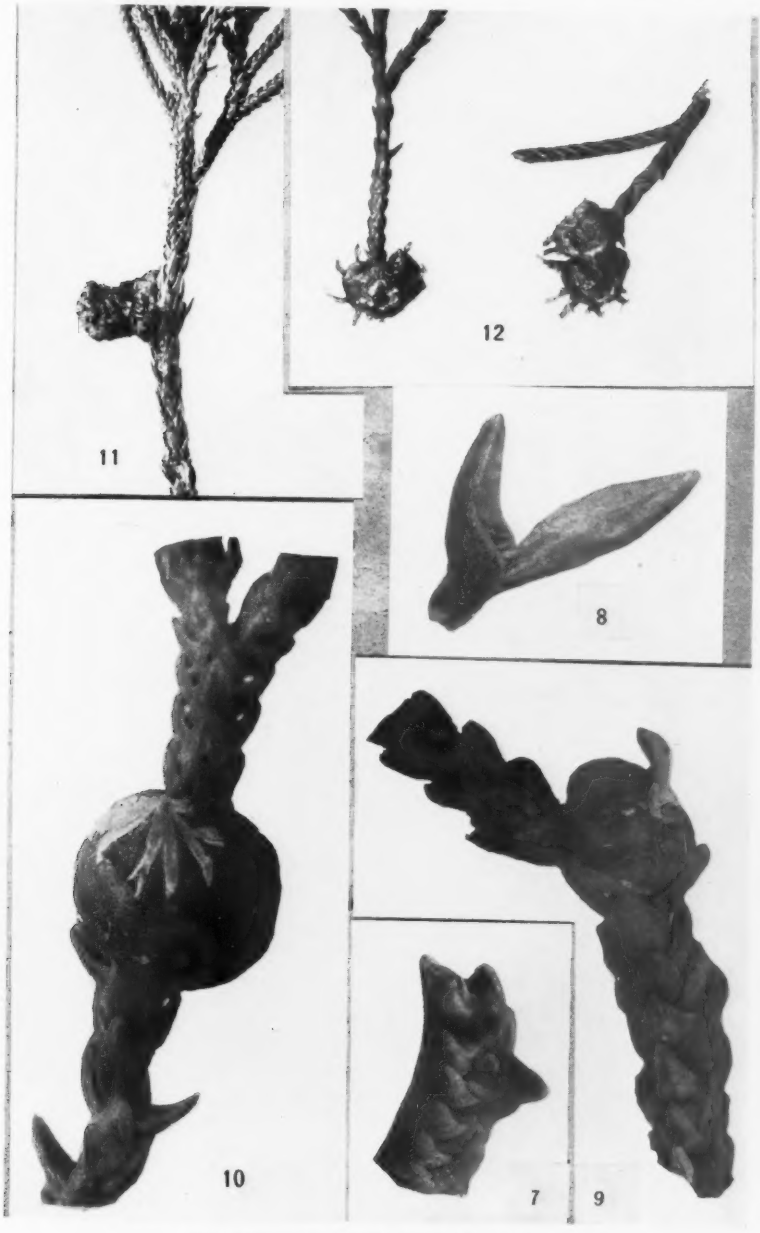
Fig. 10.—Location of two yery young galls may be noticed below larger one, which bears remains of old leaf, as in fig. 9.

FIG. 11.—Proliferated gall, portion nearest stem being alive but bearing no sori, outer portion dead and dry, and bearing remains of aecidia.

FIG. 12.—Two aecidial galls, showing protruding peridia.



THURSTON on GYMNOSPORANGIUM



THURSTON on GYMNOSPORANGIUM

PROTEIN SYNTHESIS IN CHLORELLA[1]

W. C. MUENSCHER

(WITH TWO FIGURES)

The necessity of light for protein synthesis in green plants has been questioned by a number of investigators. Few data have been presented, however, to indicate that green plants can synthesize proteins in the absence of light. It has been pointed out that fungi can synthesize proteins from organic materials when grown in the dark. Some investigators have therefore assumed that light is essential for the synthesis of proteins only in so far as it is necessary for the production of carbohydrates, which are normally supplied to a green plant through the process of photosynthesis. Experiments have been conducted in darkness in which higher green plants and algae have been supplied artificially with carbohydrates and also nitrogen in organic or inorganic combination. It seems to be well established that an abundant supply of carbohydrates or some other organic carbon source is necessary for protein synthesis in the dark. There is some indication that organic nitrogen can be utilized in protein synthesis in the absence of light. The ability of green plants to utilize inorganic nitrogen for the synthesis of proteins in the absence of light is still an open question.

In some experiments on the nutrition of several species of Chlorophyceae growing in pure cultures in the absence of light, the writer observed that some species made a considerable growth in solutions containing glucose and apparently no source of nitrogen other than that supplied in an inorganic form, such as calcium nitrate or ammonium sulphate. The investigation reported in the following pages was undertaken for the purpose of obtaining definite quantitative data on the question of whether or not green plants are able, under certain conditions, to utilize inorganic nitrogen for protein synthesis in the absence of light.

[1] Presented before the Physiological Section of the Botanical Society of America, Boston, December 27, 1922.

Literature

The evidences interpreted as indicating that green plants can synthesize proteins in the absence of light, with nitrogen present in an inorganic form only, are fragmentary and scattered; often the original data upon which statements are based are inaccessible or buried in long papers dealing with other subjects. For the purpose of discussion these evidences may be placed in three categories, as follows: (1) Increase in protein nitrogen in germinating seeds or sprouting bulbs, tubers, etc., as revealed by analyses of plants before and after growth in the dark for certain periods of time, during which carbohydrates were usually, and nitrogenous compounds sometimes, supplied under non-sterile conditions. (2) Increase in dry weight of seedlings grown in the absence of light, under sterile conditions, and in culture media containing carbohydrates and inorganic nitrogenous compounds. (3) Observations on the growth of pure cultures of algae and mosses in the absence of light on media containing carbohydrates and inorganic nitrogenous compounds. The results reported in some of the more important papers bearing directly or indirectly upon the question of protein synthesis in the absence of light, and in the presence of only inorganic nitrogenous compounds, are considered in the following discussion.

1. INCREASE IN PROTEIN NITROGEN.—Nearly fifty years ago PFEFFER (**16**) presented the idea that light influences the regeneration of proteins only in so far as it affects photosynthesis, which process supplies the carbohydrates necessary for protein synthesis in the green phanerogams. Other investigators working with higher green plants or certain detached parts thereof, ZALESKI (**26, 27**) with detached leaves of *Helianthus annuus* and bulbs of *Allium Cepa*, and SUZUKI (**22**) with etiolated seedlings of barley, concluded that protein synthesis takes place in the absence of light if sufficient carbohydrates are supplied. HANSTEEN (**9**) reported some of his own experiments in which he supplied *Lemna minor*, *Vicia Faba*, and *Ricinus communis* with various carbohydrates and nitrogen, mostly as organic compounds. After making qualitative tests, he came to the conclusion that light is probably not essential for protein synthesis in green plants. In a later paper ZALESKY (**28**) reported some experiments with stem tips of etiolated seedlings of *Vicia*

Faba floated on sugar solutions. He also analyzed sprouting tubers of *Dahlia variabilis*. In neither case did he find any direct relation between light and the percentage of protein nitrogen expressed in percentage of total nitrogen content of the plants analyzed. PALLADIN (15) reviewed the literature dealing with protein synthesis in phanerogams in the absence of light, and also discussed our knowledge of protein synthesis in general.

The conclusions of these earlier workers are open to criticism. First, in most of the earlier experiments germinating seeds, etiolated seedlings, or detached parts of mature plants, were grown in the dark with or without additional carbohydrates supplied in the solution on which they were grown. In some cases nitrogenous compounds, mostly organic, also were supplied. It is evident that under these conditions we have a plant or part of a plant containing within it complex proteins of the protoplasm, simple or reserve proteins, and possibly several other kinds of organic nitrogenous compounds in variable quantities, not to mention the various nitrogenous compounds which may come in contact with the external surface by being supplied intentionally or otherwise. It appears questionable under these conditions to interpret any increase in protein content of a plant as synthesis of proteins from inorganic nitrogen supplied to it. Second, in most of these cultures it is a fact, and in the others it is highly probable, that the plants were growing on solutions of organic substances contaminated by bacteria and fungi. Pure culture methods in the higher plants had not been developed to any degree. The assumption that plants growing on a solution containing sugar and nitrogenous compounds have necessarily utilized these substances as such hardly seems justified when the solution is contaminated by other organisms, which may first change the materials, perhaps into a more available form. While the importance of employing pure cultures in this type of work is becoming increasingly recognized at present, it cannot be over emphasized in this connection.

2. INCREASE IN DRY WEIGHT OF SEED PLANTS.—With the development of better methods of seed sterilization, an increasing number of investigators have used sterile cultures of seed plants in nutrition experiments. In a few such experiments, in which carbo-

hydrates and inorganic nitrogen were supplied, workers have obtained plants, in the dark, the dry weight of which was greater than the original dry weight of the seed used. This has been pointed out by some as evidence for protein synthesis in the dark. Since most of these experiments were concerned primarily with other phases of physiology they will not be reviewed in detail here. In table I are tabulated some examples of experiments in which plants were obtained, grown under sterile conditions in the dark with nitrogen supplied in inorganic compounds, which had a greater dry weight than the seeds from which they were produced. It must be borne in mind, however, that the figures in table I represent only those cultures in which the investigators obtained the greatest dry weight of plants; many more cultures showed a loss instead of a gain. The examples here cited are all that have come to the attention of the writer through an examination of available literature; there are probably others that might be added to the list. Table I shows that the increases in dry weight are from within the

TABLE I

SUMMARY OF RESULTS OBTAINED BY SEVERAL INVESTIGATORS GROWING SEED PLANTS UNDER STERILE CONDITIONS IN ABSENCE OF LIGHT, WITH INORGANIC COMPOUNDS SERVING AS NITROGEN SOURCES

Investigator	Plant	Growing period in days	Medium	Source of carbon	Source of nitrogen	Dry weight of seeds in mg.	Dry weight of plants in mg.
MAZÉ 12.....	Vetch	92	Solution	4% glucose	$NaNO_3$	202.8	838.2
			Solution	6% glucose	$NaNO_3$	202.8	710.0
LAURENT 11....	Zea	34	Detmer's solution	2% glucose	$Ca(NO_3)_2$	129.0	140.0
				Saccharose	$Ca(NO_3)_2$	375.0	419.0
		33		Saccharose	$Ca(NO_3)_2$	315.0	335.0
				Saccharose	$Ca(NO_3)_2$	269.0	310.0
MAZÉ and PERRIER 13.....	Zea	49	Solution	Saccharose	$NaNO_3$	500 or less	523.0
		78		Saccharose	$NaNO_3$		648.0
				Saccharose	$NaNO_3$		1093.0
KNUDSON 10....	Zea	30	Pfeffer's solution + agar	2% glucose	$Ca(NO_3)_2$ + KNO_3	296.0±10.0	307.0
BRANNON 7.....	Alfalfa	210	Pfeffer's solution	1% glucose	$Ca(NO_3)_2$ + KNO_3	2.4	17.3
				1% sucrose	$Ca(NO_3)_2$	2.4	8.0 (min.)
					KNO_3	2.4	33.8 (max.)

limits of experimental error to as great as several hundred per cent. At first sight these data seem to indicate that protein synthesis has taken place under these conditions, but in view of the relatively high protein content of the seed as compared with the plant as a

whole, especially reserve proteins, it is not impossible to conceive that the reserve protein, and possibly other forms of organic nitrogen present in the seed, are sufficient (for a time) to build the complex proteins in the protoplasmic growth of the developing seedling. To be sure, some proteins are broken down in the germinating seeds, but perhaps the decomposition products may again be utilized in synthesizing more proteins.

3. INCREASE IN GROWTH OF ALGAE AND MOSSES.—Since the first successful isolation of a green alga in pure culture by BEYERINCK (**4**), and the subsequent development of pure culture methods in the algae by this investigator and others, for example SCHRAMM (**19**), several investigators have employed pure cultures of green algae with gratifying results in physiological work. Although several papers have appeared reporting experiments with pure cultures of algae grown upon various nitrogen sources in the absence of light–(BEYERINCK **5, 6**) several species of algae on inorganic nitrogen, (RADAIS **17**) *Chlorella vulgaris* on organic nitrogen, (ARTARI **1, 2, 3**) *Stichococcus bacillaris* on ammonium nitrate and other algae on several nitrogenous compounds–the conclusions regarding protein synthesis under such conditions have usually been drawn from general observations upon these cultures and the results of quantitative analyses of phanerogams, especially those of HANSTEEN (**9**), SUZUKI (**22**), and ZALESKY (**26, 28**).

TREBOUX (**23**) reported some experiments on "pure cultures" of about twenty species of algae which were grown in the dark in various solutions containing organic acids plus ammonium sulphate or the ammonium salts of organic acids. In both cases he got as high as 50–60 mg. dry weight growth in some cultures. It is questionable whether the cultures employed were free from bacteria. DANGEARD (**8**) reported that he had succeeded in growing *Scenedesmus acutus* in total darkness for eight years on nutrient gelatine containing 1 per cent glucose and 0.08 per cent peptone. Transfers were made every two or three months. Where growth extends over such a continuous period, there is strong evidence of protein synthesis under these conditions, but this does not offer evidence for protein synthesis from inorganic nitrogenous compounds, since the nitrogen was supplied in organic combination. SERVETTAZ (**21**),

UBISCH (24), and ROBBINS (18) obtained considerable increases in growth in pure cultures of mosses grown in the dark when carbohydrates and inorganic nitrogenous compounds were supplied. ROBBINS is the only one who determined the dry weight of the growth produced under these conditions. He inoculated culture flasks with a small piece of protonema of *Ceratodon purpureus*, and got as high as 115 mg. of dry weight harvest at the end of two and one-half months. These data suggest protein synthesis under these conditions.

SUMMARY.—This brief review of the evidences for protein synthesis from inorganic nitrogenous compounds is sufficient to show that there are very few data which indicate that this process can actually take place in the absence of light. The results of earlier experiments, performed under non-sterile conditions, are questionable for the reason that under these conditions it was not determined whether the green plant actually used inorganic nitrogen in the synthesis of proteins. The evidence from pure cultures of seed plants is not conclusive. Since the seeds are usually very rich in protein, it is possible that under these conditions only the reserve protein materials were used in protoplasmic growth within the growing seedling. The evidence from pure cultures of algae and mosses is largely based upon general observations. While actual data are very few, they are suggestive though not conclusive.

Materials and methods

The organism used in these cultures was a large species of *Chlorella*, one of the unicellular Chlorophyceae. A pure culture of this species was obtained from the same strain ("no. 11") which was used by WANN (25) in his work on nitrogen fixation. The culture was known to be pure at the time of inoculation of the culture flasks. The purity of the cultures, except those in which contamination was apparent to the naked eye, was again demonstrated at the end of the experiment by microscopic examination of the harvests, and also by making duplicate transfers of algal cells upon nutrient agar slants containing 1 per cent glucose. These agar slants were incubated for a period of three weeks. In every case the algae made an excellent growth without showing any signs

of contamination by bacteria or fungi. The contaminations which did occur in two flasks could easily be detected even without the aid of a microscope.

The base solution used was a modification of the BEYERINCK solution as modified by MOORE (**14**), and found by SCHRAMM (**20**) to give good results: NH_4NO_3 0.5 gm., $MgSO_4.7\ H_2O$ 0.2 gm., K_2HPO_4 0.2 gm., $CaCl_2$ 0.1 gm., $FeSO_4$ trace, distilled H_2O 1000 cc.

With the nitrogen content of this solution as a basis, the ammonium nitrate was replaced by calcium nitrate (1.025 gm. per liter) or ammonium sulphate (0.830 gm. per liter), theoretically enough to give 175 mg. nitrogen to each case.[1] The carbon was supplied by adding 1 per cent glucose. The following series of solutions were used (see also fig. 1):

Series 1 A	$Ca(NO_3)_2$	Light cultures 13–16, 18–22
Series 1 B	$(NH_4)_2SO_4$	Light cultures 1–4, 7–10
Series 1 C	no nitrogen	Light cultures 17, 23, 24
Series 2 A	$Ca(NO_3)_2$	Dark cultures 37–43, 46, 47, 50
Series 2 B	$(NH_4)_2SO_4$	Dark cultures, 25, 26, 28, 29, 31–34
Series 2 C	no nitrogen	Dark cultures 27, 30, 35, 36
Checks	$Ca(NO_3)_2$	Cultures 44, 45, 48, 49
Checks	$(NH_4)_2SO_4$	Cultures 5, 6, 11, 12

Erlenmeyer flasks of 180 cc. capacity were used. An accurately measured volume of solution (75 cc.) was placed into each culture flask. The flasks containing the solutions were plugged with cotton and sterilized in an autoclave under twelve pounds of pressure for an hour. The rubber stoppers with continuous glass tube connections were sterilized separately. All flasks except the checks were inoculated by adding to each culture flask two drops of a dilute suspension made from a pure culture of *Chlorella* in the base solution lacking nitrogen.

After inoculation the flasks were stoppered with sterile rubber stoppers and connected by glass tubes as shown in fig. 2. The flasks were connected up into two double series, each containing two rows of twelve to fourteen flasks. The cultures were placed

[1] All chemicals used were either Baker's Analyzed or Merck's Highest Purity. The calcium nitrate was dried to constant weight by heating in an electric oven at 140°–150° C.; the ammonium sulphate was dried at 95°–100° C. Both were then considered as anhydrous.

in a basement room near two windows facing east. Series 2 was placed in an absolutely light-proof dark chamber, especially prepared for this type of work. Series 1 was placed on top of the dark

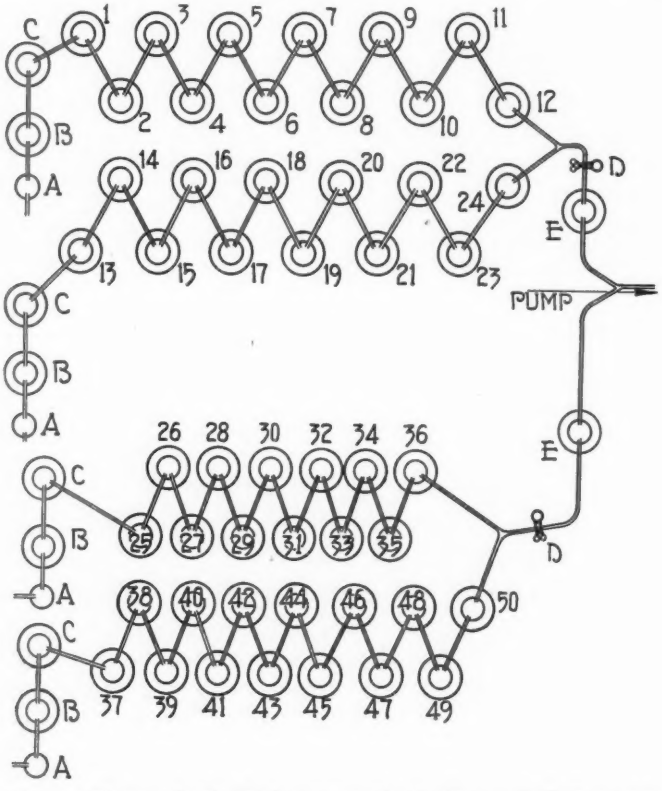


FIG. 1.—Diagram showing final arrangements of cultures: *A*, *B*, *C*, same as in fig. 2; *D*, screwcock; *E*, safety flask to catch any possible backflow from suction pump; nos. 1–24, cultures in light; nos. 25–50, cultures in dark; nos. 5–6, 11–12, $(NH_4)_2SO_4$ uninoculated check flasks; nos. 44–45, 48–49, $Ca(NO_3)_2$ uninoculated check flasks; all other numbers represent inoculated flasks.

chamber. In this cool basement room the cultures were not subject to the excessive temperatures and light intensity so common in summer. Fig. 1 shows the arrangement of all the cultures after

they were connected. The cultures were all started June 15, 1921. To make certain that the cultures of series 2 did not come under the influence of light, they were placed in the dark chamber within a few hours after inoculation, and were kept there under lock and key until the time the analyses were made. During this time the dark chamber was opened only a few times and then only at night.

The cultures were aerated with air which was first bubbled through concentrated sulphuric acid and sterile distilled water, to guard against the possibility of absorption of combined nitrogen, in the form of ammonia or nitrogen oxides, from the atmosphere

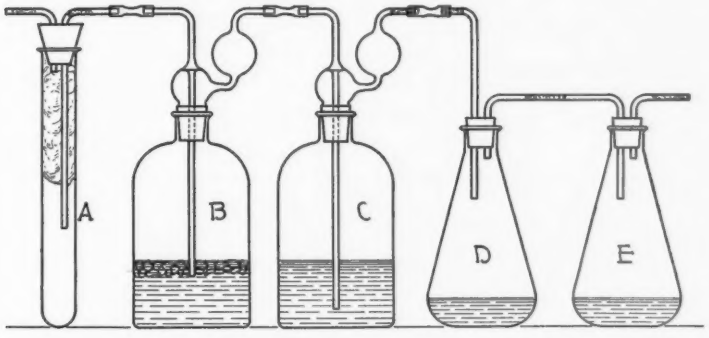


FIG. 2.—Diagram showing connection of cultures: *A*, air intake consisting of large test tube with cotton filter; *B*, wash bottle containing concentrated sulphuric acid and pumice stone; *C*, wash bottle with sterile distilled water; *D*, *E*, culture flasks.

by the organisms within the flasks. The cultures were connected in such a manner that all were aerated at the same time by a Richard's suction pump, it being necessary merely to open two screwcocks outside the chamber, thus obviating the necessity of opening the door and exposing the cultures to the light for even an instant. The aerating apparatus was first tried out with test flasks to make sure that all the connections were tight. The aerating process was carried on for from two to four hours at intervals of about three days.

Since the general appearance and condition of cultures, such as were here grown, are hard to describe and are often deceiving as regards the relative quantities of materials utilized and the amount

of growth, it was thought desirable to determine quantitatively the following data for the harvest (total growth) of the cultures: (1) total volume of the harvest per culture; (2) total dry weight of the harvest per culture; (3) total nitrogen content of the harvest per culture. The volume of the harvest was obtained by using 15 cc. glass tubes calibrated to 0.1 cc. Comparable cultures from each of the series to be compared were simultaneously centrifuged with a uniform speed of about 2500–2600 revolutions per minute. The dry weights of the harvest were obtained by filtering the algal cells directly from the culture flask, or from the centrifuge tube in those cases where the volume was first determined, on an asbestos mat in a gooch crucible of known weight. The algal cells were carefully washed several times, the crucibles and harvests dried to constant weight in an electric oven at 100° C., and the dry weight determined. The determinations of the total nitrogen content of the harvest and solutions were made by the Gunning method, modified to include the nitrogen of nitrates (**29**).

Results

All of the cultures containing combined nitrogen grew very rapidly from the beginning, as evidenced by the rapid development of green color. The intensity of the green reached a maximum in about three weeks on ammonium sulphate as the nitrogen source, after which it began to fade through various shades of green and brown, finally reaching a light brown. On calcium nitrate the intensity of the green increased for about three months; from then on this dark green color remained practically constant. In the absence of combined nitrogen very little growth took place. A slight green color was discernible from the third to the fifth day after inoculation. On the sixth day the cells began to turn orange, and a few days later they were all colorless. The growth was so slight that it could hardly be detected with the naked eye; so no attempt was made to get quantitative data from these cultures. In table II are recorded some of the notes taken on the various cultures, mostly at the conclusion of the experiment, 235 days after inoculation.

In tables III and IV are given the data secured from the cultures which were analyzed September 30, 1921, three and one-half months after inoculation. The data presented in table III show that when calcium nitrate was used as the nitrogen source, the total dry weight of the harvest of cultures grown in the light and in the dark was practically the same. The individual dry weight determinations checked very closely in both the light and dark cultures. The

TABLE II

NOTES ON CULTURES AT END OF EXPERIMENT

Series of cultures	Color of cells		Condition of chromatophores	Diameter of cells	Remarks
	Individual	In mass			
	Light				
A. $Ca(NO_3)_2$...	Bright green	Black-green	Large, clathrate	3–16 μ diameter, rather uniform, mostly below 8μ	Starch present; all cells on bottom and sides of flask
B. $(NH_4)_2SO_4$..	Light brown	Brown	.....	10–40 μ diameter, mostly 10–20 μ	Starch present; all cells on bottom of flask
C. $-N_2$.....	None	None	.....	.....	Cells turned orange on sixth day; soon became colorless
	Dark				
A. $Ca(NO_3)_2$...	Bright green	Olive green	Apparently broken into several	8–120 μ diameter, very variable, mostly 30–40 μ	Some cells forming clumps; cells on bottom and sides of flask, some suspended; starch present
B. $(NH_4)_2SO_4$..	Brown	Brown	.....	10–80 μ diameter mostly 30–40 μ	All cells on bottom of flask; starch present
C. $-N_2$.....	None	None	.....	.....	.....

average dry weight of the harvest in the light was 254 mg., while in the dark it was 252 mg. A comparison of the light cultures with the dark shows that the volume of the latter is somewhat greater. Here again the individual volume determinations of similar cultures vary but slightly. The difference in the volume of the harvests in the light and dark cultures was probably due to the fact that in the dark cultures the algal cells were larger and stuck together in lumps, so that they did not pack as firmly as smaller separate cells. The total nitrogen content of the harvest of the

light cultures was about 11 mg., as compared with about 8 mg. for the dark cultures.

The data here given represent accurate measurements of three criteria of growth, namely, total dry weight, total volume, and

TABLE III

VOLUME, DRY WEIGHT, AND TOTAL NITROGEN CONTENT OF HARVEST OF PURE CULTURES OF *Chlorella;* SOURCE OF NITROGEN $Ca(NO_2)_3$; DURATION OF GROWTH 106 DAYS

SERIES 1 A, IN LIGHT				SERIES 2 A, IN DARK			
No. of culture	Dry weight of harvest in gm.	Volume of harvest in cc.	Total nitrogen content of harvest in mg.	No. of culture	Dry weight of harvest in gm.	Volume of harvest in cc.	Total nitrogen content of harvest in mg.
13.....	0.2420	1.45	.....	37.....	0.2445	1.60	7.84
14.....	0.2582	1.40	11.90	39.....	0.2545	1.50	.....
15.....	0.2585	1.25	11.58	40.....	0.2511	1.60	7.80
16.....	0.2645	1.20	11.23	41.....	0.2595	1.60	8.82
18.....	0.2475	1.25	.....	42.....	0.2501	1.55	.....
Average...	0.2541	1.31	11.57	Average..	0.2519	1.57	8.15

TABLE IV

VOLUME, DRY WEIGHT, AND TOTAL NITROGEN CONTENT OF HARVEST OF PURE CULTURES OF *Chlorella;* SOURCE OF NITROGEN $(NH_4)_2SO_4$; DURATION OF GROWTH 106 DAYS

SERIES 1 B, IN LIGHT				SERIES 2 B, IN DARK			
No. of culture	Dry weight of harvest in gm.	Volume of harvest in cc.	Total nitrogen content of harvest in mg.	No. of culture	Dry weight of harvest in gm.	Volume of harvest in cc.	Total nitrogen content of harvest in mg.
1.....	0.0625	0.31	.....	25.....	0.0425	0.20	1.26
2.....	0.0570	0.30	1.54	26.....	0.0480	0.23	1.12
3.....	0.0650	0.32	1.54	28.....	0.0420	0.25	0.92
4.....	0.0685	0.32	1.66	29.....	0.0396	0.21	.....
Average ..	0.06325	0.3125	1.58	Average..	0.0430	0.2225	1.10

total nitrogen content. A comparison of the cultures in the light and dark with reference to these three criteria leaves no doubt as to the ability of *Chlorella* to utilize calcium nitrate for growth in the absence of light. In just what form the nitrogen is utilized, or how much of it is used in synthesizing proteins was not deter-

mined. Qualitative tests of water extracts of dry algal cells gave no indication of the presence of nitrates. Since the only source of combined nitrogen available to the algal cells was nitrate, and an abundant growth (over 251 mg. per culture) resulted in the presence of calcium nitrate, while no growth took place in the absence of combined nitrogen, this is positive evidence that the nitrate is used by this organism in the absence of light. This evidence is further strengthened by the fact that large quantities of nitrogen, about 11 mg. in the light and 8 mg. in the dark cultures, were absorbed per culture from the solution and found in the algal cells in other than nitrate forms. The disappearance of nitrate nitrogen and the large growth of algal cells represents a large turnover of nitrate nitrogen into protein nitrogen. One cannot escape the conclusion that protein synthesis has taken place in the dark. How otherwise could the inoculum, a few algal cells in two drops of nitrogen-free solution, build up enough protoplasm to supply a quantity of cells represented in a volume of 1.5 cc. or in 251 mg. of dry weight? It is believed that these data offer positive and conclusive evidence of the ability of *Chlorella* to carry on protein synthesis from inorganic nitrogen in the absence of light.

The data in table IV show the results obtained with ammonium sulphate as the source of nitrogen; these are self-explanatory and need not be discussed in detail. The values obtained for total dry weight, total volume, and total nitrogen content were much lower than when calcium nitrate was used as a source of nitrogen. In general, according to the preceding criteria of growth, ammonium sulphate is at least to some extent utilized by *Chlorella* in the dark. The evidence, however, is not as striking and conclusive as with calcium nitrate.

Most of the cultures were continued for about eight months, until February 8, 1922, when they were finally taken down and analyzed. The results obtained with these cultures are given in tables V and VI. Volume determinations of the harvest were not made because the previous volume determinations did not reveal any striking differences between the light and dark cultures. Table V shows the results obtained from the cultures containing calcium nitrate as the source of nitrogen. The analyses of the harvests and the remaining solutions show that most of the available

TABLE V

DRY WEIGHT AND NITROGEN DETERMINATIONS OF PURE CULTURES OF *Chlorella*, IN MG. PER CULTURE, SOURCE OF NITROGEN $(CaNO_3)_2$; DURATION OF GROWTH 235 DAYS

No. of culture	Dry weight of harvest	Total N content of harvest	Total N content remaining in solution	Total N content of harvest + solution	Total N content of solution
	Series 1 A, in light				
19	259.2	11.90	1.26	13.16	.....
20	246.0	11.20	1.68	12.88	.....
21	244.0	11.76	1.40	13.16	.....
22	227.0	10.74	1.52	12.26	.....
Average	244.05	11.40	1.465	12.865	13.17*
	Series 2 A, in dark				
43	263.5	8.54	2.10	10.6	.....
46	229.6	9.10	2.66	11.7	.....
47	243.0	8.26	2.92	11.18	.....
50	252.5	9.10	3.64	12.74	.....
Average	247.15	8.75	2.83	11.555	13.17*

* This equals the calculated amount of nitrogen. Some difficulty was experienced in recovering all the nitrogen from the uninoculated checks containing calcium nitrate.

TABLE VI

DRY WEIGHT AND NITROGEN DETERMINATIONS OF PURE CULTURES OF *Chlorella*, IN MG. PER CULTURE, SOURCE OF NITROGEN $(NH_4)_2SO_4$; DURATION OF GROWTH 235 DAYS

No. of culture	Dry weight of harvest	Total N content of harvest	Total N content remaining in solution	Total N content of harvest + solution	Total N content of solution
	Series 1 B, in light				
7	64	1.40	11.48	12.88	.....
8	58	1.58	11.48	13.06	.....
9	61	1.59	11.22	12.81	.....
10	67	1.64	10.24	11.84	.....
Average	62.5	1.553	11.11	12.65	13.17*
	Series 2 B, in dark				
31	47	1.26	11.48	12.74	.....
32	50	1.40	11.76	13.16	.....
33	44	1.40	11.44	12.84	.....
34	41	1.12	12.02	13.16	.....
Average	45.5	1.295	11.675	12.975	13.17*

* This equals the calculated amount of nitrogen. The amount actually recovered from the checks was 13.23 mg. of nitrogen per culture.

nitrogen had been used up, only about 1 mg. of the 13 mg. supplied in the original solution remaining. The dry weight of the harvest did not change appreciably after the first analyses, which were made 105 days after inoculation, indicating that most of the growth had taken place before that time.

Table VI shows the results obtained from the cultures containing ammonium sulphate as the source of nitrogen, 235 days after inoculation. These almost exactly duplicate those presented in table IV obtained from similar cultures 105 days after inoculation. The analyses show that very little of the nitrogen, here supplied as ammonium sulphate, had been used by the alga. Most of the nitrogen, about 11 mg. out of the 13 mg. supplied, was still in the solution. Since the dry weight of the harvest remained practically the same in both the light and dark cultures, it is evident that growth in these cultures ceased long before they were analyzed. *Chlorella* seems to be able to utilize slightly more nitrogen in the light than in the dark. This difference is more marked when calcium nitrate rather than ammonium sulphate is used as the source of nitrogen. This is indicated not only by the greater nitrogen content found in the harvest, but also by the smaller quantity of nitrogen remaining in the solution in the light cultures when compared with the dark cultures. It is difficult to account for this difference. Various factors may have been modified when some of the cultures were placed in the dark. It is possible that the rate of respiration was altered. In the light cultures additional carbohydrates were probably formed by photosynthesis, while the dark cultures received only the carbohydrates supplied by the solution (750 mg. of glucose per culture). These additional carbohydrates may have been sufficient to account for the increased amount of nitrogen utilized by the cultures provided with calcium nitrate. Analyses of the remaining solutions showed that practically all the glucose was used up in the calcium nitrate solution, but large quantities remained in the ammonium sulphate solution.[2] This

[1] The following data represent the quantity of glucose remaining in the solutions at the end of the experiment. Duplicate determinations were made by ALLIHN'S method of two cultures from each series. Calcium nitrate in light, less than 1 mg. glucose per culture; calcium nitrate in dark, less than 1 mg. glucose per culture; ammonium sulphate in light, 310 mg. per culture; ammonium sulphate in dark, 290 mg. per culture.

would seem to indicate that carbohydrate supply does not account for the slightly increased amount of ammonium sulphate utilized by the light cultures.

The data presented in the tables show that, under the conditions of this experiment, when similar cultures were supplied with equal amounts of nitrogen (13 mg.), in one case supplied as nitrate by calcium nitrate and in the other as ammonia by ammonium sulphate, much more growth occurred (approximately 250 mg. dry weights of cells), and much more nitrogen was utilized (approximately 11 mg. in the light and 8 mg. in the dark) when the nitrogen was supplied as a nitrate than when supplied as ammonia. Only about 63 mg. of dry weight were produced in the light and 44 mg. in the dark, while the amount of nitrogen utilized was about 1.5 mg. in the light and 1.2 mg. in the dark cultures, when supplied as ammonia. These data do not necessarily indicate the relative availability of nitrate nitrogen and ammoniacal nitrogen, nor of calcium nitrate and ammonium sulphate, for the organism here used. It was not intended to determine the relative availability of the different forms of nitrogen used. It may be pointed out, however, that when the calcium nitrate was replaced by ammonium sulphate, various changes may have taken place within the solution. It may be that the ammonium sulphate solution did not support a better growth on account of its high acidity. The calcium nitrate solution was slightly alkaline.[1] Some workers have stated that algae grow best in a solution that is nearly neutral or slightly alkaline. This preference, however, probably varies with different species.

Since WANN (**25**) used this same organism, "*Chlorella* no. 11," on mineral nutrient agar with 1 per cent glucose (the nitrogen supplied as calcium nitrate) and obtained 36 per cent free nitrogen fixation in 235 days, it was thought of interest to check up the results of this experiment to see whether any fixation of free nitrogen

[1] The H-ion concentrations of the culture solutions were determined at the end of the experiment by the use of CLARK's colorometric indicators. Duplicate cultures were taken from the $Ca(NO_3)_2$ series and from the $(NH_4)_2SO_4$ series in the dark, and three determinations were made of each culture. The $Ca(NO_3)_3$ solutions were found to be somewhat alkaline (average, 7.7 P_H), while the $(NH_4)_2SO_4$ solutions were strongly acid (average, 4.6 P_H).

had occurred. An examination of tables V and VI shows that the sum of the nitrogen found by analysis of the harvest and the remaining solution in no case is greater than the calculated amount of combined nitrogen originally supplied. These data do not seem to indicate that *Chlorella* sp. can fix free nitrogen under the conditions of these experiments. WANN, however, grew all of his cultures on the surface of agar, while the writer employed only solution cultures; the results therefore are not comparable.

Summary

1. One of the unicellular Chlorophyceae, *Chlorella* sp., was grown in pure culture in a nutrient solution to which combined nitrogen was supplied in inorganic combination, either as calcium nitrate or ammonium sulphate.

2. Cultures were grown in diffuse light and in the total absence of light for 105 or 235 days.

3. Quantitative determinations were made of the volume, dry weight, and total nitrogen content of the growth obtained.

4. The data obtained offer strong evidence indicating that *Chlorella* sp. can synthesize proteins in total darkness when nitrogen is supplied in inorganic combination.

5. No evidence of free nitrogen fixation by *Chlorella* sp. was obtained.

This work is the outcome of a problem suggested by Professor J. R. SCHRAMM, to whom the writer is indebted for advice, encouragement, and a constant interest during its progress. The pure culture of the organism used in this work was kindly supplied by Dr. F. B. WANN. The writer wishes to thank other members of the department who have given assistance in various ways.

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DELAYED GERMINATION AND CATALASE ACTIVITY IN XANTHIUM

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Introduction

It has been known for some years that the dimorphic seeds of species of *Xanthium* differ in germination behavior, and that the delayed germination of the upper seeds is caused by certain coat and embryo characters which act together in effecting this delay. The coats of the upper seeds are somewhat less pervious to oxygen than are those of the lower seeds, and at the same time the embryos of the upper seeds must have a greater partial pressure of oxygen to initiate growth. As a result of these differences, the uppers may be kept in germinators for months without germinating, whereas the lowers usually show under identical conditions complete germination in a few days. The upper seeds become more active in the germinators, their carbon dioxide output increases, but the rate of metabolism does not reach the level necessary for germination.

Experiments demonstrating these differences have been repeated many times by students in our laboratories during the last ten years, with fairly uniform results. Occasionally a careless student will have a high percentage of uppers germinate; but more careful repetitions practically always demonstrate the delayed germination of the uppers, and the overcoming of the delay by any means that introduces sufficient oxygen to the embryos, by breaking the coats, by use of increased partial pressures of oxygen, or by use of dilute hydrogen peroxide solutions. It has been one of the most satisfactory laboratory experiments for clear cut results in our experience.

In a recent paper MCHARGUE (7) claims that he was unable to demonstrate any such differences between the upper and lower seeds of *Xanthium*. He collected seeds in the spring, planted them in pots of soil kept at 28° C., and obtained rather prompt germination of about 80 per cent of the upper seeds. He concludes that "there is no inherent property contained in the embryos of the

small (upper) seeds of this species, which is the cause of delayed germination, neither is there any evidence that the seed coat interferes in any way with germination." He did find delayed germination of the uppers, however, when the seeds were left in the burs. Noting the persistence of the bur about the upper seed, a fact which CROCKER (5) also observed and noted, MCHARGUE attributes the delay wholly to the bur.

We have often observed, as did ARTHUR (4) many years ago, that the beaks of *Xanthium* burs are left open, through the shriveling of the style, sufficiently to admit oxygen to the seed cavity. Moreover, it must be remembered that the work of CROCKER (5) and SHULL (9) was done largely with seeds removed from the burs, and that in all this work the observed delay could not be associated in any manner with bur characters. In nature, as CROCKER points out, independent variability in the rate of decay of bur and seed coats leads to considerable variability in the time of germination of the two types of seeds, but our delayed germination studies have eliminated this bur factor.

In view of MCHARGUE's statement regarding the lack of physiological differentiation of the two seeds, it is worth while to summarize briefly the differences observed, and to add some new evidence of differences in enzyme equipment of upper and lower seeds. MCHARGUE's results will receive critical consideration later.

SUMMARY OF DIFFERENCES

One of the important differences discovered by CROCKER was the difference in the minimum temperature for germination, which is modified by the amount of oxygen the embryo can obtain. In the case of the upper seeds, three temperature minima were worked out, as follows:

In moist germinator, coats intact, ordinary atmosphere, about 33° C.
In moist germinator, coats intact, atmosphere of oxygen, about 22° C.
In moist germinator, coats broken, ordinary atmosphere, about 18° C.

The lower seeds also have different minima for germination with coats intact, and with coats broken:

In moist germinator, coats intact, ordinary atmosphere, about 23° C.
In moist germinator, coats broken, ordinary atmosphere, about 18° C.

It is evident that the difference which exists between uppers and lowers as long as the coats are intact disappears completely when the coats are broken on both seeds. The cause of this temperature difference was traced by experiment to slight differences in the rate of absorption of oxygen by the two seeds. Since in both cases the coat is the limiting factor in oxygen absorption, the coats of the uppers must be less permeable to oxygen than the coats of the lower seeds.

Studying the naked embryos, SHULL found that the minimum demand of the uppers for oxygen is fully twice as great as in the lowers for the initiation of growth, if the temperature is sufficiently high. Thus at 31° C. the uppers require about 7 mm. of oxygen pressure, the lowers only 3 mm.; at 21° C. the difference is not so great, the uppers requiring about 12 mm., and the lowers 9.5 mm. for germination. This larger oxygen need must be supplied through a less permeable coat, embryo and coat acting jointly in producing the conditions that lead to delay. In respiration tests it was found (10) that upper seeds with coats intact respire about 40 per cent as much in the germinators as the lowers with unbroken coats. If the coat respiration is considered equal in the two types of seeds, the respiration of the upper embryos must be less than 25 per cent of that of the lower embryos, principally because the coats of the uppers restrict oxygen diffusion, for when the coats are removed, the respiration of equal masses of upper and lower embryos at equal (atmospheric) oxygen pressures is nearly equal. Both seeds respire when placed in moist germinators, at an accelerated rate; but the uppers receive their oxygen supply too slowly to permit the attainment of a rate of energy release requisite for the rapid metabolism of germination and growth of the seedling. The uppers may lie, therefore, on moist cotton for months with coats intact, respiring all the while, but failing to germinate.

Catalase activity of Xanthium seeds

ARTHUR (4) first suggested that the enzymes of the two seeds might differ in amount or activity. He had in mind the digestive enzymes, and relative food supplies. CROCKER, however, was unable to detect any differences in digestive activity of extracts of

the upper and lower seeds. As the physiological differences of significance in the delay center about the utilization of oxygen, the respiratory enzymes are important in this connection. From the work of APPLEMAN (**1, 2**), CROCKER and HARRINGTON (**6**), and others it has been established that there is a closer correlation of respiration with catalase activity than with the activity of any other oxidizing enzyme. ATWOOD (**3**) has recently found that changes in catalase activity and respiratory activity in wheat treated with formaldehyde run parallel. The reason for this close correlation is not yet known. In some respects catalase does not act like a true enzyme, but whether it is one of a series of causal factors, or whether it is one of the products of respiration, the close correlation of catalase activity with respiratory activity enables one to use the former as a rough measure of the latter in cases where the correlation is known to hold. In *Xanthium* the two do run parallel, apparently.

During the last germination season the writers were engaged in a study of the catalase activity of upper and lower seeds of *X. pennsylvanicum* as taken from the field. We were particularly interested in quantitative differences in catalase activity of the two types of seed, and in any changes in catalase activity during the germination period. Tests were made at intervals of seven days from late March to the middle of May, at which time many seeds had already germinated in the field.

The preliminary studies showed very marked differences in catalase activity of uppers and lowers, the latter showing 155–206 per cent greater activity than the former, after allowing for the difference in weight of upper and lower seeds. This method of making calculations is not very accurate, since catalase activity is not strictly proportional to the quantity of material used. We are indebted to CROCKER for some unpublished data on the catalase activity of *Xanthium* seeds. The seeds were collected at the Virginia end of Long Bridge, across the Potomac, October 14, 1918, and used a week later. Seventy upper seeds and as many lowers, coming from the same burs, were ground fine and run through a 100-mesh sieve, and equal quantities of powder used in each test. The results are shown in table I.

Dry storage of the seeds for a month or two, either in the bur or cut from it, ground or unground, produces little change in catalase activity. The enzyme from these seeds, therefore, is relatively

TABLE I

CATALASE ACTIVITY OF *Xanthium* SEEDS; CUBIC CENTIMETERS OF OXYGEN LIBERATED BY POWDERED SUBSTANCE

TIME IN MINUTES	LOWERS				UPPERS			
	0.1 gm. powder		0.2 gm. powder		0.1 gm. powder		0.2 gm. powder	
	I	II	I	II	I	II	I	II
1	9.3	9.0	18.0	18.7	7.5	7.4	15.2	15.6
3	20.1	19.7	37.0	36.9	16.7	16.6	32.2	32.3
5	25.7	25.3	44.2	44.2	21.5	21.3	39.0	38.9
10	31.0	30.5	.....	.....	25.4	25.7	.....	.....

stable under storage conditions, at least during the early months of storage. This is shown clearly in the data of table II, which were obtained with seeds stored from October 14 to December 9–12, 1918.

TABLE II

CATALASE ACTIVITY OF FRESH AND STORED LOWER SEEDS; CUBIC CENTIMETERS OF OXYGEN LIBERATED BY 0.2 GM. POWDERED SUBSTANCE

TIME IN MINUTES	IN BURS		CUT OUT, UNGROUND		GROUND	
	I	II	I	II	I	II
1	15.8	16.0	15.5	15.0	15.5	18.0
3	33.0	35.5	32.0	32.0	35.5	35.7
5	39.5	44.2	38.3	37.8	42.7	42.3

If the seeds are stored in germinators, however, but kept at temperatures below the minimum for germination, both lowers and uppers show increases in catalase activity and in respiratory intensity. The data for catalase are shown in table III for seeds with coats on, kept in germinators for 50 days but not germinated. The activity in table III should be compared with that found with 0.2 gm. of the powdered substance in table I. These data show very clearly that there is a difference in catalase activity of upper and lower seeds, the latter showing considerably greater activity. These results have been confirmed by the writers in a

long series of determinations under various conditions. The differences observed are in harmony with the more rapid use of oxygen by the lower seeds, and with the differences in germination behavior between these and the upper seeds.

TABLE III

CATALASE ACTIVITY OF *Xanthium* SEEDS IN GERMINATORS; CUBIC CENTIMETERS OF OXYGEN RELEASED BY 0.2 GM. POWDERED SUBSTANCE

TIME IN MINUTES	UPPERS				LOWERS			
	20° C.		Ice chest		20° C.		Ice chest	
	I	II	I	II	I	II	I	II
1.....	21.0	21.5	21.7	23.0	18.0	18.0	20.0	18.0
3.....	39.0	39.5	43.3	44.5	33.0	32.7	37.2	38.0
5.....	46.2	46.8	51.0	52.0	39.0	40.0	44.5	45.3

This study of the catalase activity of seeds of *X. pennsylvanicum* during the germination season was made with seeds which had weathered in the burs from the ripening season until used. A single group of plants near the Hull Botanical Laboratory furnished all the seeds used. The burs were collected at weekly intervals, and seeds were used only from burs that contained two well formed seeds. From about 30 to 50 seeds were used for each test, the lowers and uppers being ground separately after drying. The powdered substance was passed through a 100-mesh sieve, and 0.2 gm. of the powdered seeds was used with 10 cc. of "dioxygen," precautions being taken to neutralize the acids of the seeds and reagent. The catalase determinations were made immediately after grinding, and in all cases the oxygen liberated has been reduced to standard conditions, 0° C., 760 mm. The data are shown in tables IV and V for the lowers and uppers respectively.

During the first several weeks there is little change in the catalase activity, a slight decrease being noted in both lowers and uppers, but beginning with the minimum activity on April 12, at which time the earliest germinations in the field were observed, each succeeding collection until the last shows a marked increase in the activity of the lowers. By May 10 most of the germinations in the field had occurred, and the collections were probably made up of seeds of

less than average activity. In the case of the uppers, the changes during the active germination season are much less pronounced. Indeed, with the exception of the duplicate readings of May 5, there is practically no increase. The uppers taken on May 10 have slightly less activity than those first collected March 28. These data seem to indicate, therefore, that in nature the difference

TABLE IV

CHANGES IN CATALASE ACTIVITY OF LOWERS OF *X. pennsylvanicum* DURING GERMINATION SEASON; CUBIC CENTIMETERS OF OXYGEN LIBERATED BY 0.2 GM. OF POWDERED SUBSTANCE

TIME IN MINUTES	DATES OF COLLECTIONS													
	March 28		April 5		April 12		April 19		April 26		May 3		May 10	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II
1	17.7	19.0	16.9	15.5	17.0	15.7	20.6	21.8	22.4	21.8	22.3	20.7	26.6	25.8
3	34.7	34.7	32.2	31.1	29.7	29.8	37.5	37.9	39.9	39.9	42.8	40.1	45.6	44.6
5	39.7	39.2	37.6	37.1	33.6	34.2	44.0	44.0	46.4	46.2	51.7	48.8	52.0	50.8
10	42.6	42.0	41.4	41.8	35.6	37.0	49.1	49.3	52.2	51.9	59.9	56.8	56.1	55.1

TABLE V

CHANGES IN CATALASE ACTIVITY OF UPPERS OF *X. pennsylvanicum* DURING GERMINATION SEASON; CUBIC CENTIMETERS OF OXYGEN LIBERATED BY 0.2 GM. OF POWDERED SUBSTANCE

TIME IN MINUTES	DATES OF COLLECTIONS													
	March 28		April 5		April 12		April 19		April 26		May 3		May 10	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II
1	13.2	12.6	10.8	10.1	9.8	9.5	10.4	10.2	11.1	10.4	12.6	13.1	12.8	12.4
3	25.3	24.4	21.1	19.6	18.8	18.6	20.4	20.4	21.2	19.7	25.6	25.6	23.4	22.9
5	28.5	28.0	24.6	22.9	21.5	21.5	24.6	24.6	25.4	23.1	31.9	31.5	26.6	26.5
10	30.5	30.0	26.8	24.8	22.9	23.2	28.0	28.3	28.8	26.0	37.7	37.1	28.9	28.9

which normally exists between uppers and lowers is greatly increased as germination proceeds, during the first spring after ripening.

In addition to the field studies, some determinations have been made with individual seeds and seedlings during germination in Petri dishes. Seeds of about the same dry weight were chosen for duplicate tests, but due to variations in the rate of development and to natural variations in activity, the results are quite variable.

The seeds or seedlings were ground with sand and $CaCO_3$ in a mortar, and the fine material used as in the other tests. The volumes of oxygen are all reduced to standard conditions. In table VI is shown the catalase activity of the lowers, in air dry condition, after soaking in water on ice for 22 hours, and in germinators at 25° C. for 22, 48, 72, and 88 hours following the soaking.

It is unfortunate that a change of dioxygen was necessary for the determinations on seeds kept in the germinators. The Oakland Chemical Company claim that their product is fairly stable, and that the loss in strength should not exceed 5 per cent per year during use. From the data it is evident that the catalase activity

TABLE VI

CATALASE ACTIVITY OF LOWERS OF *X. pennsylvanicum* DURING GERMINATION; CUBIC CENTIMETERS OF OXYGEN LIBERATED BY INDIVIDUAL SEEDS

Time in minutes	Air dry		Soaked on ice 24 hours		In germinator 22 hours		48 hours		72 hours	88 hours	
	Weight in mg.										
	59.6	59.6	55.4	55.0	62.0	62.4	60.0	59.4	50.0	57.4	57.6
	I	II	I	II	I	II	I	II	I	I	II
1	6.3	6.6	5.2	5.3	32.3	14.2	69.4	35.5	50.8	73.9	85.0
3	12.9	12.9	10.5	10.9	50.3	28.5	83.0	57.7	73.2	89.7	130.1
5	16.3	16.7	14.0	14.6	57.3	36.4	.....	65.3	78.3	.....	146.8
10	21.6	21.7	18.2	18.9	62.0	42.8	.....	71.7	84.0	133.0	162.1
Length of hypocotyl	.....	.....	.....	.....	10 mm	Coat broken	30 mm	2 mm	13 mm	23 mm	30 mm

of both lowers and uppers increases in the germinators when cut from the burs, but the lowers greatly exceed the uppers in increase, except in case of actual germination of uppers, as in column 11, table VII. In general the data agree with those of table III furnished by CROCKER. A slight decrease in activity was noted in those kept on ice for 22 hours, but the number of determinations was small, and the difference may have no significance.

There is some evidence indicating that long exposure of the uppers in the germinators at temperatures below the minimum for germination with intact coats, leads to lowered vitality, low vigor of germination, or induced dormancy. The catalase activity of such seeds is found to have fallen, following the initial increase

in activity, until it is no greater than in air dry seeds. The data in table VIII compare the activity of air dry upper seeds with that of seeds which have been in the germinator for 2, 43, 51, and 93 days respectively.

TABLE VII

CATALASE ACTIVITY OF UPPERS OF *X. pennsylvanicum* DURING GERMINATION

TIME IN MINUTES	AIR DRY		SOAKED ON ICE 22 HOURS		IN GERMINATOR 22 HOURS		48 HOURS		72 HOURS		88 HOURS	
	Weight in mg.											
	58.2	59.0	55.2	55.0	55.0	55.2	53.0	54.8	50.0	50.0	48.0	48.0
	I	II	I	II	I	II	I	II	I	II	I	II
1	5.8	3.6	3.6	3.4	7.5	6.5	14.6	22.3	21.2	43.7	22.0	16.8
3	11.9	7.4	7.5	7.0	15.6	12.5	26.8	39.9	38.1	64.0	42.6	30.0
5	16.0	10.1	10.3	9.7	20.2	16.2	31.8	45.0	44.4	70.2	51.9	34.4
10	21.0	13.7	13.5	13.0	23.6	19.5	35.0	48.7	47.5	74.8	58.4	37.0
Condition of coats					Unbroken	Unbroken	Unbroken	Unbroken	Unbroken	Hypocotyl 11 mm long	Unbroken	Unbroken

TABLE VIII

CATALASE ACTIVITY OF UPPERS OF *X. pennsylvanicum*, AIR DRY, AND FOR SHORT AND LONG PERIODS IN GERMINATOR; CUBIC CENTIMETERS OF OXYGEN LIBERATED BY INDIVIDUAL SEEDS

TIME IN MINUTES	AIR DRY		IN GERMINATOR 2 DAYS		43 DAYS		51 DAYS		93 DAYS	
	Dry weight in mg.									
	58.2	59.0	53.0	54.8	54.9	51.3*	40.0	38.7	43.3	41.8
1	5.8	3.6	14.6	22.3	7.6	23.9	2.5	7.9	5.5	3.4
3	11.9	7.4	26.8	39.9	12.7	37.1	5.1	15.7	12.1	7.4
5	16.0	10.1	31.8	45.0	14.1	40.1	7.2	20.0	17.0	10.8
10	21.0	13.7	35.0	48.7	15.0	42.6	11.2	24.1	23.5	15.7

* This seed was broken open May 10, after 42 days in germinator. It germinated in 12 hours, and had a hypocotyl 5 mm. long 24 hours after breaking the coat. Data are for the seedling at this stage.

In these experiments, the first few days in the germinator produced an increase in catalase activity; but as the period of exposure lengthened, we found decreasing activity to about the same level as in seeds which have been kept in dry storage. Then if the coats of these seeds are broken, so that oxygen becomes available, there is again a very rapid increase in catalase activity, as

shown in column 7 of table VIII. This decreased activity is not shown in the data of table III, although the earlier activity was not determined in that case. A considerable number of determinations have been made on such seeds, and the results have all been consistent.

Two of the sets of seeds, 51 and 93 days, are lighter than the rest in table VIII. At the beginning of the long exposure they weighed about as much as the others, but the original weights were not kept. They lost weight by slow respiration, and the given dry weights were calculated from the wet weights at the time the catalase was determined in these last two cases.

Discussion

No attempt has been made in this preliminary work to follow accurately the respiration changes, or to determine the respiratory quotient during the long period in the germinators. The few data on record merely show that so far as we have gone, changes in respiration rates are accompanied by similar changes in catalase activity. Miss SHERMAN (8) found that catalase activity increases in *Crataegus* seeds over a long period of after-ripening. The seeds of *Xanthium* have no after-ripening, and it seems very probable that catalase activity and respiratory activity rise and fall together in the case of *Xanthium* seeds during delayed germination.

The main purpose of this work has been to test anew the behavior of *Xanthium* seeds, in view of MCHARGUE'S failure to obtain delay. The basis of his rejection of delayed germination is an experiment in which upper seeds, removed from the burs and planted in soils, germinated to 80 per cent in a few days. The other 20 per cent remained ungerminated for 90 days, and these he calls non-viable, although he does not state how he determined that they were non-viable. Since CROCKER'S tables show no data carried beyond 33 days, these experiments were run for longer periods to demonstrate the correctness of CROCKER'S conclusions. This work was done with the assistance of Mr. W. J. CRIBBS, who carefully prepared and kept 26 seeds properly moistened in the germinator for 90 days. In table IX is shown the behavior for lower and upper seeds under identical conditions.

One seed of the uppers germinated at the end of 30 days, and no others during the remaining 60 days. Two of the delayed seeds from this set were chosen for the catalase tests in table VIII; the

TABLE IX

PERCENTAGE GERMINATION OF LOWERS AND UPPERS OF *X. pennsylvanicum*

Time in days	Lowers	Uppers
2	10	0
5	90	0
6	100	0
30		4
90		4

remainder were carefully broken open to determine whether they were still viable. The germination, after 90 days in the germinator, is shown in table X.

TABLE X

GERMINATION OF DORMANT UPPERS OF *X. pennsylvanicum*

Time in days	Percentage germination
1	0.00
2	4.33
3	13.00
4	17.33
5	30.33
6	56.33
7	69.33
8	78.00
9	86.66
11	91.00
12	95.33
19	100.00

The germination is much less vigorous than in uppers cut from the burs, removed from the testas, and set to germinate at once, but the mere fact that seeds do not germinate for 90 days is not sufficient evidence of non-viability. The low vigor of germination seen in table X is consonant with the low catalase activity of seeds long in the germinators, as shown in table VIII.

The high percentage of non-viability in MCHARGUE'S work is very unusual for weed seeds. CROCKER (5) has called attention

to the high viability of weed seeds, and all who work with such seeds must be impressed with the low mortality ratio among them. In our long experience with *Xanthium* seeds, the number of non-viable seeds in material less than 3 to 5 years old would be considerably less than 1 per cent. We have had a higher percentage perish under severely adverse conditions, but not in normal germination. There is a small fly, *Tephritis aequalis* Loew, which parasitizes the cocklebur seed, but most frequently the seeds are completely destroyed, and such burs are eliminated when one chooses seeds only from burs with two sound seeds. The writers believe it highly probable that, unless tests were actually made to prove that the seeds were dead, the 20 per cent of uppers classed as non-viable by McHARGUE were exhibiting the very delay which he claims does not occur.

As to the reasons why he did not find the normal percentage of delay in the uppers, one can only surmise; but there are several things which may have influenced his results. The seeds he used were collected in the field in the spring after freezing and thawing repeatedly in the changeable climate of Kentucky. Such freezing and thawing would tend to increase the natural defects which SHULL has found to occur in the coats of *Xanthium* seeds, and thus would enable a larger percentage to secure enough oxygen for germination. Another factor which might have some influence is the temperature. McHARGUE used 28° C., which is not so very far below the minimum which CROCKER found for the upper seeds of certain species. This temperature minimum is not necessarily the same for the uppers of all species, and the minimum may have been exceeded in this case. LLOYD has suggested the possibility that certain races of cockleburs may arise which do not possess coat differences in uppers and lowers. While the possibility must be admitted, the writers have never found any material which failed to show these differences when tested. Finally, one must always be careful in removing the seeds, not to injure the coats. When dormancy is produced by coat characters, and the embryo itself is not dormant, one may readily bring about considerable germination by rubbing the seeds a little, or roughly handling them, even though the coats may not appear to be injured. This has been tested in the case of *Xanthium*.

Table XI shows the germination behavior of uppers taken from the same material as the uppers of table IX, but handled somewhat less gently in extracting them from the burs. The coats had not been cut, nor could injuries be seen on careful inspection. Nevertheless, the germination is considerably hastened. During the experiment several of the seeds were used for catalase determinations, but the percentages given are based on the number of seeds at the start.

TABLE XI

GERMINATION OF UPPERS OF *X. pennsylvanicum* AFTER LESS CAREFUL HANDLING

Time in days	Percentage germination
2	0.0
7	15.0
8	20.0
23	35.0
25	40.0
32	50.0
34	65.0
37	75.0

In conclusion, we would like to emphasize the fact that no one has claimed that the upper embryo of *Xanthium* is dormant. It has no after-ripening period. It will germinate at once if the temperature is above the minimum, if the oxygen pressure is above the minimum, if oxygen is supplied in hydrogen peroxide, or if the coat is broken. It is only when perfect unbroken coats cut down the rate of diffusion of oxygen below that meeting the embryo's need for germination, that delay results. The differences found between uppers and lowers as to catalase activity are in harmony with the other data on record, and would tend to make possible a delayed germination of the upper seed.

Summary

1. The upper seeds of *Xanthium* show constantly less catalase activity per unit of dry substance than the lower seeds.

2. Catalase activity is relatively stable in dry stored seeds, for several months.

3. Stored in imbibed condition in germinators below the temperature for germination, the catalase activity of both uppers and lowers increases immediately following exposure in the germinators.

4. In the field during the germination season, lower seeds increase in catalase activity, while the uppers lag behind them, showing practically no increase.

5. In the laboratory, soaking seeds on ice seems to depress activity slightly, but during subsequent germination, catalase activity increases *pari passu* with the germination. Upper seeds show much less increase than lowers, except when they actually germinate.

6. Long delay of germination while in the germinators leads first to increased catalase activity of uppers, followed by a decline, which at the end of several months leaves the seeds with no more activity than air dry seeds. The decreased catalase activity accompanies a decreased vigor of germination.

7. The catalase differences in *Xanthium* seeds are in harmony with other physiological differences, which cooperate to bring about delayed germination of upper seeds with intact coats.

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TORSION STUDIES IN TWINING PLANTS. II

H. V. HENDRICKS

(WITH TEN FIGURES)

Introduction

Since the publication of the first paper on this subject,[1] the work has been extended and considerable data accumulated. In order to make the present communication clear, a brief description of the method and apparatus used in the previous work is included here.

The problem of pure torsion (without coiling) was studied in *Phaseolus multiflorus* and *Tiniaria Convolvulus* by means of a modified form of auxanometer. It was shown that as the internode grows in length at first it twists slowly, later more rapidly, and at the end of growth in length there is generally a slight but variable reverse twist. The rigidity, or resistance to twisting, of the stem was shown to increase gradually with lignification (tested microchemically by means of hydrochloric acid and phloroglucin) until length is nearly completed, when there is a sudden upward turn in the rigidity curve. References to other points will be made later. The apparatus, in the main, is illustrated in fig. 1. The lower part of the internode used is secured in a special clamp (*cl*) held on the arm of a larger sliding clamp (*K*), while the upper part is secured in a clamp in a light frame (*F*). This is suspended by a silk thread running over wheels on top of the apparatus, and the proper tension, generally a little more than enough to prevent coiling, is given by a weight (*Wt*). A second sliding clamp (K^1) carries a semicircular scale (*Sc*) and a telescope (*T*). By means of these and two pairs of mirrors at right angles in the upper part of the frame, the torsion is measured in degrees. A loose thread from the frame to the top of the apparatus (not shown) gives by its coils the number of complete revolutions of twist.

The increase in length was formerly measured by means of the scale (Sc^1) and a double pointer. Recently, however, another device

[1] HENDRICKS, H. V., Torsion studies in twining plants. BOT. GAZ. 68:425–440. 1919.

was adopted which has been found accurate enough for most purposes (within about 0.5 mm.), and in some ways more satisfactory. A rod (R) with a metric scale is held on pivots so as to be easily rotated about a vertical axis. A second telescope (T^1) is secured to a clamp that is adjustable anywhere on this rod. Since any part of the vine may be viewed with this telescope, the growth in length, not only of the entire internode but of marked parts also, may readily be observed.

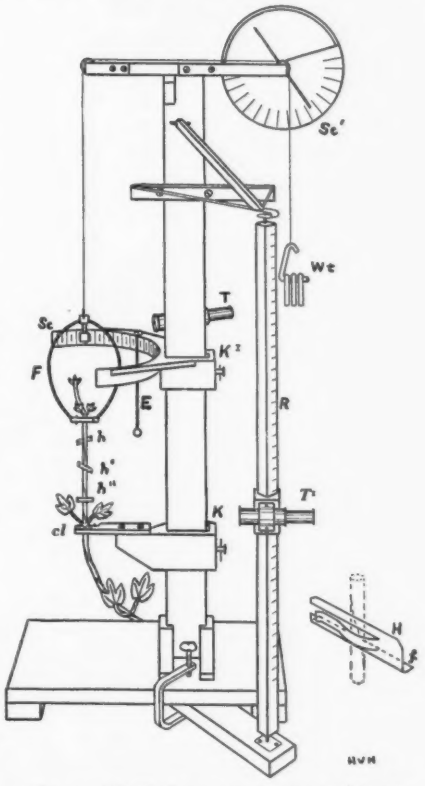


FIG. 1.—Modified auxanometer for studying torsion and certain other features in twining plants.

Another useful device is made of sheet aluminum. It is about 3×0.5 cm. in size, and is cut as a clip (H) with a flange (f), and weighs about 0.1–0.15 gm. Several such may be attached with a little pressure to the stem as shown in fig. 1 (h, h', etc.). A hatpin (E) with the point in a small cork is hung over the edge of the scale (Sc). An angle reading at any such place on the internode is made after bringing the scale to the proper level and then moving this hatpin into line with the flange on the clip. Previously I described a more exact but heavier and more complicated "mirror clamp," but these aluminum clips seem to be accurate enough for most purposes.

Attention is called to the previous definition of the term "rigidity." It is the relative resistance to twisting through a unit angle

of an average unit length of vine. An estimate of this value is given by L/T^2, where L is the length in centimeters of the part between the clamps, and T is the time of oscillation in seconds of the frame (*F*). For a more complete explanation, and for a definition of the term "coefficient of rigidity," which takes into account diameter, the reader is referred to the previous article.

New data

The general behavior of the species studied, *Humulus Lupulus* and *Ipomoea purpurea*, is presented first. Fig. 2 shows that the

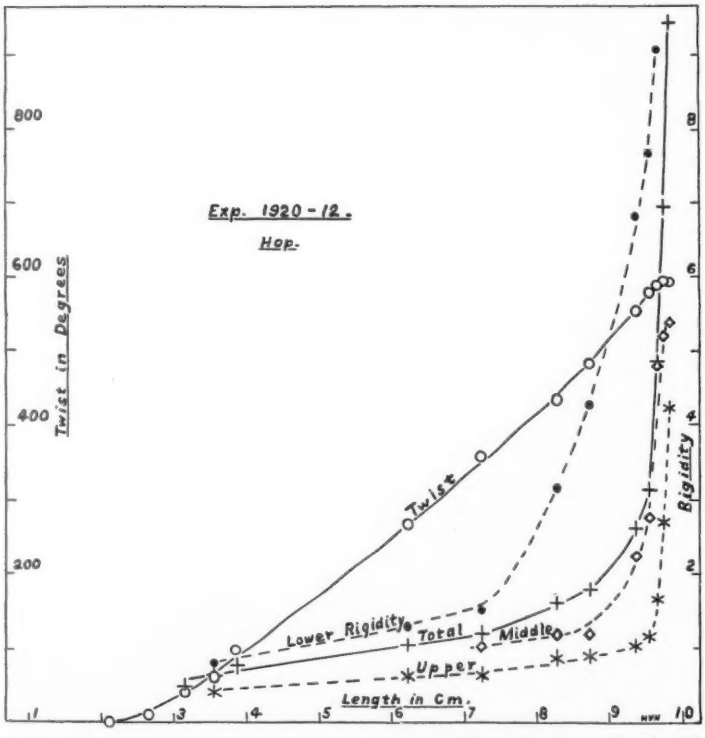


FIG. 2.—Experiment with hop, showing: 1, general laws of twiners when in this apparatus with reference to (*a*) growth in length and twist and (*b*) increase in rigidity, or resistance to twining (see curve "Total"); 2, that lowest part of internode attains its extensive rigidity before middle and this before uppermost part; this increasing rigidity corresponds partly to increasing lignification and partly to increasing diameter; tension equals 10 gm.; final average diameter equals 1.8 mm.

hop obeys the same general rules as the flowering bean and the bindweed previously mentioned. The morning glory shows the same behavior so far as rigidity is concerned, but in many cases (fig. 3) there is in the middle of the twist-curve somewhat of a convexity (a "hump") where a concavity would be expected. This point will be referred to later. Microchemical tests show that lignification accompanies the development of rigidity in these two species in about the same way as in those studied before.

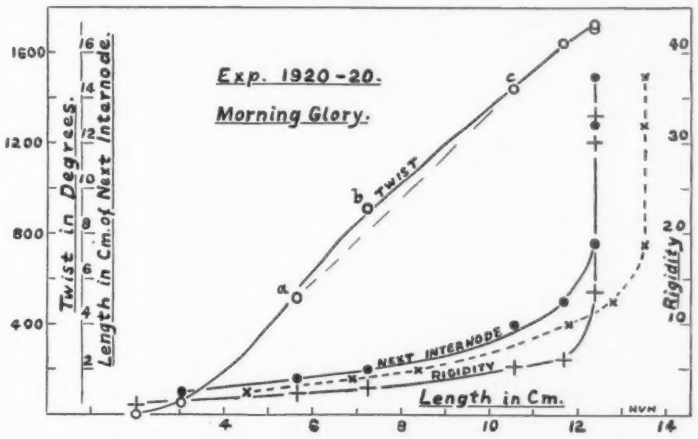


FIG. 3.—Experiment with morning glory, showing that the general laws apply here also, except that the twist-curve has a "hump" (*a*, *b*, *c*), accompanied by a slight coil in stem; reverse twist (final drop), here only 2°, has been exaggerated; growth of next internode in length, when compared with growth in length of part held between clamps shown to follow law similar to that of rigidity; tension equals 20 gm.; final average diameter equals 2.1 mm.

TWIST BY PARTS.—A few observations on this phase of the subject were noted in the previous paper. Further experiments have been made, and fig. 4 illustrates one of them. Several aluminum clips were attached to an internode of the hop and used as previously indicated. Curves I–V (heavy lines) show the succeeding increase in length and in twist of points (marked with the clips) that were at first 1.1, 2.1, 2.9, 3.7, and 4.5 cm. respectively, from the lower clamp. The dotted lines *AA'*, *BB'*, *CC'*, *DD'*, *EE'* are drawn through groups of points on these curves representing

simultaneous readings. Consider the time indicated by the dotted line *CC'*. By referring first to the line *BB'*, we see that at the mark I the stem has stopped growing in length and in twist, and has acquired a slight reverse twist. Strictly speaking, the point *e* should be marked as simultaneous with points on *CC'*, but this would lead to confusion. At the next mark II the stem has attained full

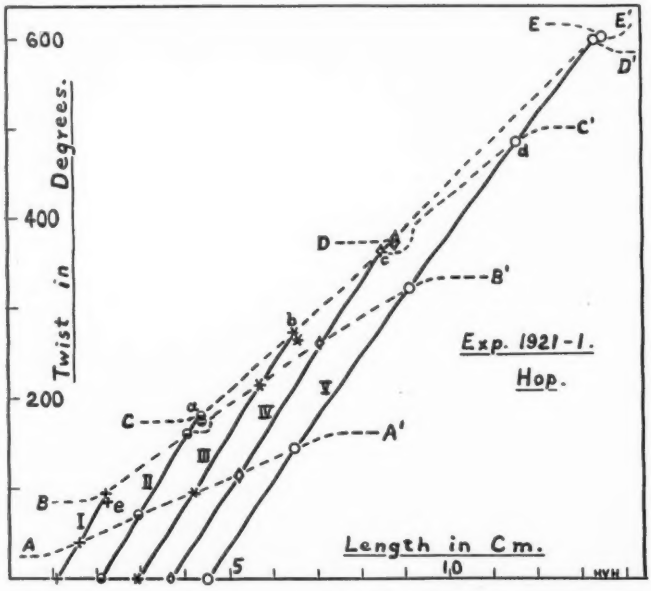


FIG. 4.—Experiment with hop, showing that completed homodromous torsion, followed by slight reverse, occurs successively along internode from below upward; heavy lines, growth in length and torsion of various marks on stem; dash lines, drawn through simultaneous readings, except points of reverse twist; tension equals 10 gm.; final average diameter equals 2.05 mm.

length and twist, also at the mark III (as indicated by points *a* and *b* on *CC'*), while at the marks IV and V it is still growing (points *c* and *d*). The line *DD'* shows that at the next reading only at the top of the internode is the stem still increasing in length and twist. The dotted line *EE'* passes through only one point; at the time represented by this all the other marks on the stem have acquired reverse twists. If the experiment had been continued, a reverse

twist would probably have been noted in curve V, for the top of the internode, and probably another reading between *BB'* and *CC'* would have shown that at the mark II length and twist were completed before they were at mark III. It is seen, therefore, that completion of growth in length and torsion, followed by a slight reverse twist, occurs successively and gradually from the proximal to the distal end of the internode. Similar results were obtained with the "mirror clamp" in the flowering bean. An actual demonstration in the case of the bindweed has not been attempted on account of its small diameter, and in the morning glory it has not been attempted in quite so careful a manner on account of the heavy growth of downward projecting hairs. It seems reasonable to assume, however, that the same general results would have been found. Curves I–V, it will be observed, do not show at the beginning a distinctly less slope than later, but this is due to the fact that the internode had attained considerable length before the experiment was begun.

RIGIDITY BY PARTS.—Rigidity has been described as a measure of relative resistance to twisting with reference to average unit length. This term "average" is significant, because the value obtained, as well as the amount of lignification as tested chemically, is not the same throughout the whole internode during its growth. This point will be discussed later. In experiment 1920–12 (fig. 2), this question was studied in the case of the hop quite carefully. Every time readings were made, after noting length, angle, and period of oscillation of the total vine used, it was released from the clamps, and, with the lower, middle, and upper parts clamped separately, the corresponding length (L) and period of oscillation (T) of each part were determined. The internode was then replaced in the clamps in nearly the same position as before, and the length and angle were again carefully noted, so that the increase in length and torsion could be obtained the next time. Rigidity in each case was calculated from the formula L/T^2. Fig. 2 shows the rigidity curves for the three parts and for the whole, each time the total internode being taken as abscissa for all of the values of rigidity. It is observed that the lower part reaches its sudden increase in rigidity before the middle part, and this before the upper part,

while the corresponding values of the lower part are greater than those of the upper part (accounted for to some extent by differences in diameter). The values of rigidity for the vine as a whole are seen to be something like averages for the values of the parts. Similar results were obtained in case of the morning glory. It is thus demonstrated that the development of rigidity proceeds gradually during the growth of the internode from below upward. This is due in part to the gradually increasing diameter of the stem from below upward, but, as shown for the bean in the previous paper, is also due to increase in lignified tissue.

COMPARISON OF INTERNODES.—In many experiments on the hop and on the morning glory when the usual readings were made, the length of the internode above the one held in the upper clamp was measured. One such experiment for morning glory is given in fig. 3, where lengths of the upper internode and values of rigidity of the part held between the clamps are plotted as ordinates with corresponding lengths between clamps as abscissae. It is evident that the curves are similar. In fact, by choosing the proper units, curves could be drawn that would almost coincide, at least in this particular experiment. The dotted curve shows that comparison of the upper internode with the total internode in the clamp gives the same general rule.

DIAMETER STUDIES.—Growth in diameter was frequently studied incidentally with the vine held in the apparatus. Other diameter studies were made with the vine free from the apparatus and without support. Diameter measurements were made with a device similar to a *V*-wire gauge, as many readings as possible being taken along the internode each time. Fig. 5 shows such an experiment (without the apparatus) in the case of the morning glory. Lengths and average diameters are represented in one case by half-shaded circles for one internode and by stars for the internode next beyond. It is seen that curve I fits quite readily all these points. Another branch of the morning glory was similarly observed at the same time, and curve II is drawn through the points (circles and crosses) obtained. While this does not fit these points as well as in the case of curve I, there is still a tendency for the same rule to apply. It is noticed that in each case the average increase in diameter is

most rapid at first, but that throughout most of the growth the graph is almost a straight line, so that increase in average diameter is throughout most of its growth practically proportional to increase in length of internode. Similar results were obtained in the studies on the hop, bindweed, and flowering bean. This applies to average diameters of course. Before growth in length was complete, it was found that at any one time there was gradual diminution in the diameter readings from below upward, but that as a rule this was most marked in the very upper part of the internode studied. Average diameters, in experiments with the apparatus, were found to increase in about the same way, but the readings at any one time

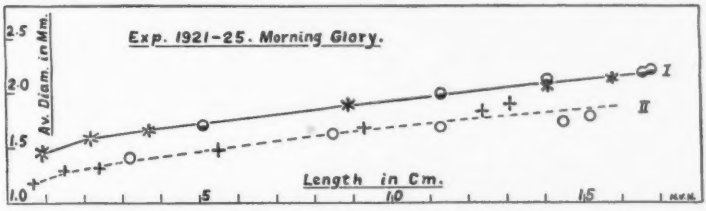


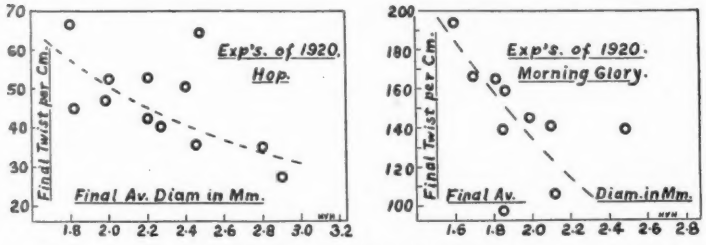
FIG. 5.—Study of growth in diameter of morning glory, without apparatus; increase in average diameter nearly proportional to increase in length of internode during most of growth; curve I for two successive internodes on one branch, and curve II for another branch; there is often a tendency for points on two successive internodes to fit same graph, as in I.

as a rule did not show as much tapering from the proximal to the distal part.

COMPARISON OF TWIST AND DIAMETER.—The former article mentioned incidentally that the dimensions of the usual curves obtained depended on the final diameter. In fig. 6 this is shown graphically for experiments on the hop performed in 1920. Final twist per unit length is plotted vertically against final average diameter horizontally. That the various points cannot be made to touch even approximately any simple curve is evident, but in a general way it is seen that there is a tendency for the final twist per centimeter to vary inversely with the final average diameter. Omitting the upper right hand point, and applying the method of least squares to the other data, it was found that $y = 115 \div x^{1.19}$ as the resulting equation, where $y =$ final twist per centimeter and

x = final average diameter. As a matter of fact, the dotted curve is drawn in accordance with this, but no importance can be given to it at present. A series of experiments on the flowering bean in 1918 yielded a similar group of points. Fig. 7 shows about the same thing for experiments on the morning glory in 1920. The dotted curve is drawn here without previous mathematical treatment. It is noticed that the relative divergence of points from this curve is about the same as in the other.

COILING EFFECT WHILE UNDER TORSION.—Mention has already been made of the fact that the twist-curve for morning glory frequently shows a convexity in the middle accompanied by a slight coiling in the stem. In experiment 1920–20 (fig. 3) such a curve is



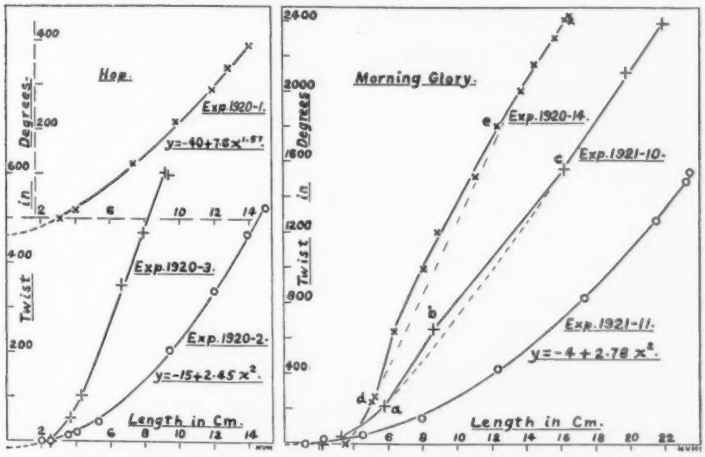
FIGS. 6, 7.—Series of experiments with hop and morning glory; in each case there can be seen only a general tendency for final twist per unit length to vary inversely with final average diameter.

shown. At point a the internode used showed a little coil of about one turn and about 2 mm. in diameter, also at point b, but at point c this had practically disappeared. In this case a tension of 20 gm. was used throughout. In some experiments an extra weight was added when coiling appeared. The fine dash lines in fig. 3 represent about the curve which theoretically should have been obtained. Experiments 1921–10 and 1920–14 (fig. 9) show about the same phenomenon. It was often difficult to use enough weight to prevent coiling without causing slipping or breaking. Occasionally an experiment was performed with morning glory with a negligible amount of coiling, and in the curve practically no irregularity of the kind just mentioned. Experiment 1921–11 (fig. 9) is an example of this. In some cases, after the coiling had practically disappeared from the lower part, it appeared in the upper, another illustration of the fact that the various processes involved move steadily

upward in the growing internode. This coiling was also observed in the other species studied, but to a much less extent.

Mathematical studies

In a number of cases the data on torsion and length have been investigated mathematically. Consider experiment 1920–2 of the hop (fig. 8). By use of the method of least squares, with y taken as twist in degrees and x as length in centimeters, it was found that the relation between them could be expressed by the following empirical equation: $y=-15+2.45x^2$. The curve is drawn in



FIGS. 8, 9.—Fig. 8, twist-curves for hop: experiment 1920–1, tension 15 gm. very little reverse twist (final drop), curve not a true parabola; experiment 1920–2, tension 10 gm., final drop 2° (not shown), curve practically a parabola; experiment 1920–3, tension 10 gm., final drop 7°, curve not a parabola. Fig. 9, twist-curves for morning glory: experiment 1920–14, tension 7 up to *d*, later 10 gm., final drop 9° (exaggerated); "hump," with some coil in stem, between *d* and *e* (probably not enough tension); experiment 1921–10, tension 25, later 30 gm., very little final drop; "hump" (*a*, *b*, *c*) in curve accompanied by some coil in stem, no coil beyond *c;* experiment 1921–11, tension 20 up to second point, later 30 gm., final drop 4° (not shown); almost no coil, curve practically a parabola.

accordance with this. The broken line indicates the course which the twist theoretically followed during the first 2 cm. of the internode's growth. Another equation was worked out: $y=-18+2.64x^2-0.0129x^3$, but this does not fit the actual data much, if any, better. In the case of the morning glory a similar equation, $y=-4+2.78x^2$, was determined for experiment 1921–11 (fig. 9).

This fits the data on the whole with about the same percentage of accuracy as the equation just mentioned for the hop. Each of these equations expresses a parabola, in which it can readily be shown that the rate of increase in y with respect to increase in x at any point is simply proportional to x; hence the rate of twisting at any time with reference to rate of growth in length at that time is proportional to the length already attained. It has been shown that through most of an internode's growth in length the increase in average diameter is nearly proportional to the increase in length. If this is also true of the actively twisting part at any time, it would then follow that rate of twisting is merely proportional at any time to the diameter attained by the growing part. Should this be found to be a general rule, it might be a principle of considerable importance. Unfortunately, however, in most of the cases investigated mathematically this is not exactly the case, for example, experiment 1920–1 (fig. 8). The equation $y=-40+7.6x^{1.57}$ here expresses the relation between twist and length, but it is not a true parabola, in that the exponent of x is not equal to 2. By simple inspection of the curve for experiment 1920–3, by comparison with the other two curves in fig. 8, it is seen that this also is not a true parabola. The data in a number of other experiments were investigated in this way, and after careful inspection and comparison of all the curves it appears probable that to most of the experimental data the parabola does not apply.

Discussion

The slight reverse in torsion represented by the final drop at the top of the twist-curve, as already mentioned, has been noticed in all of the four plants studied. On the average this has been greatest in the morning glory, occasionally as much as 60°, and least in the hop. The amount has varied a great deal and occasionally very little was observed. This was probably due, in part at least, to an insufficient number of readings, so that the highest point was missed. It is suggested that this slight reverse in torsion may be due to the newly formed lignified tissue. In the hop and morning glory, as well as in the flowering bean and bindweed, slight irregularities after completion of growth in length and torsion have been noticed. These consist in backward and forward readings of a few degrees in twist, and of 0.1–2.0 mm. in length (usually less than

1.0 mm.), as indicated by the scale and double pointer. Variations in the length of the silk thread, due to changes in moisture, account for these variations in length of internode only in part. No further explanation is attempted at present.

A few wilting experiments were carried on, in the main as follows. After the vine was put into the apparatus and observed for a certain length of time, the stem was cut below the lower clamp. Increase in length ceased, but changes in torsion continued, sometimes homodromously (in the same direction as the twining in that species), sometimes antidromously (in the opposite direction). The amount and rate seemed to depend on the extent to which lignification had taken place. Unfortunately, no rule was found governing the direction of twist during wilting. If it could be shown, for example, that this always occurs in one direction, this might prove to be another principle of considerable importance. A few further studies were made on antidromous torsion, but without any significant results, and I am not in position to modify the explanation usually given.

The question may be asked, to what extent does this apparatus interfere with natural forces at work in the stem of the twining plant? Of course the coiling proper and the antidromous torsion, which are usually perfectly natural features, are not seen in this apparatus. Attempts were made to study in detail homodromous torsion in stems that were free from the apparatus as well as from ordinary supports. It was difficult to do this satisfactorily, but casual observations seemed to show that the same general laws apply here also. At the end of the experiment it was frequently noticed that there was a relative enlargement of the stem just under the upper clamp, although not enough to be seen readily except by measurement. This is probably caused by more or less interference with the upward stream of sap due to the clamp. I see no reason, however, for believing that this weakens the general conclusions drawn.

The slight "coiling" sometimes observed during studies in torsion, especially in the morning glory, deserves further discussion. That it occurs before much of the tissue has been lignified and while the diameter is relatively small, and that the coil withstands considerable tension, I believe are quite important observations. In

experiments where a small tension was used there was a tendency for the coil to remain as growth proceeded, but it became less noticeable. Perhaps this is only an expression of excessive torsion. It was observed, however, that when a considerably coiled stem of morning glory or hop was subjected to further tension in the apparatus, there was an increase homodromously in the angle reading. The same was noticed in the case of a steel spiral spring studied in the same way. It is possible that it may be shown later to be a factor connecting, more or less, simple torsion with circumnutation and twining proper. I do not feel certain that the "hump" in the curve can always be correlated definitely with "coiling"; neither this nor other minor irregularities can readily be explained.

Table I compares in a general way some of the outstanding features of the plants that have been studied. The terms dextrorse

TABLE I

Species	Direction of twining and of homodromous torsion	Cross-section	Final average diameter (mm.)	Final twist per cm.	Usual tension (gm.)	Coiling under tension
Black bindweed...	Left	7-angled	1.0–1.4	46–104	5	Very slight
Scarlet runner bean	Right	2-grooved	1.5–2.3	66–140	10	Very slight
Hop.....	Left	6-angled	1.8–3.0	28–69	10	Very slight
Morning glory.....	Right	Circular with abundant hairs	1.6–3.5	60–194	20–40	Noticeable

and sinistrorse are avoided, since they seem confusing to the writer. The terms right and left are used instead, in the same sense as right and left-handed screw threads. Final diameter means average diameter at the end of growth in length, or soon afterward.

It is suggested that the ribs on the hop and bindweed interfere with twisting, and so, taking diameter into account, make their twists per centimeter less than would be expected in stems of about the same size of the other two species.

It is admitted that the method described for studying this problem is not ideal, and that the apparatus is crude in some respects, but it seems reasonable that, with this method of approaching the problem, a more exact apparatus would have yielded no

better results, and that the conclusions given are justifiable. In each experiment only relative values are obtained, for the most part only with reference to that particular experiment. At present it does not seem worth while to try to express rigidity, etc., in absolute units, so that the values obtained for these plants could be compared with similar values obtained by physicists for inorganic material. One reason for this is that the stems are not uniform in texture. Another difficulty is that, in defining rigidity, it cannot be said that the term "average" is used exactly in its ordinary sense, since in the case of an actively growing stem the upper, less rigid part may have more effect on the period of oscillation than the lower, more rigid part.

Each experiment lasts a few days, and readings have usually been made twice a day. More frequent readings would have been desirable. A great need in this problem is some practicable device for controlling external conditions, including temperature, air-moisture, and, so far as possible, illumination. With such a device it would probably be possible to compare more satisfactorily final average diameters and final twists per unit length. It is desirable to carry on further mathematical investigations, but a device like the one suggested seems necessary before this can be done to advantage. It is probable that other irregularities might largely be avoided in this way also. The experiments have been carried on in the greenhouse, and the light, except direct sunlight, has been nearly the same on all sides. It would be well to study the light relations in this problem in more detail, using perhaps a clinostat.

It must be understood that no special emphasis is placed on the rigidity studies presented here, because they do not demonstrate anything really new. As a matter of fact they were undertaken incidentally, while an attempt was made to measure the actual forces of torsion. Nevertheless, it is believed that they are of some value. With this method I have demonstrated about the same thing in the scape of *Plantago lanceolata* as in the experiments already given, and it is probable that the same general conclusions would be found to apply to other non-twiners, so far as rigidity is concerned. I suggest that the various methods may find more or less application to other problems, certain studies in wilting, for example.

Finally, it should be borne in mind that all studies in growth must ultimately be referred to cell activity. This is the case in the studies presented here, where advantage has merely been taken of a peculiarity of certain plants. Further investigations in the physics, histology, and microchemistry of these plants are required, however, before such references can be made with advantage, and before the phenomena involved can be explained.

Summary

The flowering bean, bindweed, hop, and morning glory have been studied with reference to torsion when the stem is free to twist, but under enough tension to prevent almost all coiling. It has been demonstrated that the following phenomena occur, and most of them are illustrated schematically in fig. 10.

1. Completion of growth in length and torsion, followed by a slight but variable reverse in torsion, occurs successively in the internode from below upward.

2. Torsion with reference to length proceeds slowly at first, later more rapidly. This is true of all points on the internode.

3. The relation between torsion and length has been investigated mathematically to some extent, and in some experiments the resulting curve is nearly a parabola.

4. Average rigidity of the stem as a whole, due largely to lignification, proceeds slowly at first, but rapidly when growth in length is nearly complete, while actual lignification proceeds successively from below upward. This is probably the case with non-twiners also.

5. During most of growth in length, rate of increase in average diameter with reference to increase in length is practically constant, and there is often a tendency for this rate to be the same in two adjacent internodes.

6. The next internode increases in length slowly at first with reference to the first internode, but rapidly when the first has attained nearly its full length. This is similar to development of average rigidity.

7. Slight coiling (not shown in diagram), accompanied by a "hump" in torsion-curve, was sometimes observed, especially in

the morning glory even when under considerable tension. This largely disappeared as growth proceeded.

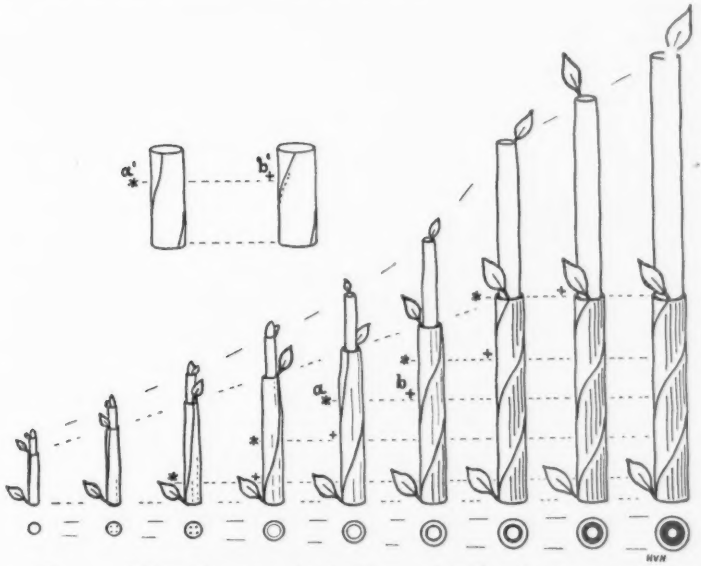


FIG. 10.—Schema showing main phenomena of torsion in twiners: spiral lines indicate torsion, shading indicates rigidity and lignification, circles indicate average cross-sections, shading of circles indicates average rigidity; after each point marked * no further growth in length or torsion occurs for that place on stem; note straight dotted lines to right; at points marked + slight reverse twist has occurred (illustrated at a', b' in smaller figure for points a, b in main figure); for sake of clearness reverse twist not shown in main figure; in this schema total twists and lengths are taken so that they follow practically the law of parabola.

8. There is a general tendency (not shown in fig. 10) for final twist per unit length to vary inversely with the final average diameter, but this rule is only a rough one.

I am again indebted to Mr. PETER J. KLAPHAAK, now of the United States Department of Agriculture. He has examined the literature for me, but has found nothing new that seems to bear on the problem.

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PERMEABILITY OF THE CELL TO ELECTROLYTES

ORAN RABER

When one seeks for an explanation which will fit all the experimental data brought forward in the studies on the permeability of the living cell to salts, he finds many difficulties. The methods employed by different investigators, the materials used, and the conditions under which the experiments were performed are so diverse that it would seem almost impossible to find any common solution. Numerous explanations have been suggested to explain some of the effects of electrolytes. A few may be outlined briefly.

Among the first theories to explain the action of neutral salts was the hydration hypothesis of FREUNDLICH (2), who called the action "lyotropic." According to this theory the action of the salt is upon the solvent, and varies with the water attracting power of the salt. This seems to explain particularly well such phenomena as the lowering of the freezing point of water. It will be recalled that the nitrate lowers the freezing point less than the sulphate in equimolar concentrations, and that the SCN, Cl, Br, and I come in between the two. The more water taken up by the salt, the less there would be available in the free state; and the greater the attraction between the salt and the water, the harder it would be to separate it out by freezing. The action of the salts in the precipitation of colloids as found by HOFMEISTER (5) would be similarly explained. The citrates and sulphates, according to this theory, take so much water from the solution in which the egg albumen is suspended that the albumen becomes precipitated out. On the other hand, the chloride and nitrate, having less attraction for water, do not remove as much from the solution, and the albumen remains in suspension. It has been shown by the writer (17) that in *Laminaria*, when a series of sodium salts is used, the changes in permeability produced by the salts are in general as in the HOFMEISTER series, the salts causing an increase in permeability in the following order: $SCN < I < Br < NO_3 < Cl <$ acetate $< SO_4 <$ tartrate $< PO_4 <$ citrate. If the "lyotropic" explanation were to be applied

to these results, it might possibly be done by saying that the changes in permeability were due to the precipitation of the colloids in the plasma membrane. The citrate being more hydrophile than the sulphocyanide attracts more water from the solution or suspension of substances in the plasma membrane, resulting in their precipitation. This would diminish the resistance of the membrane to the ions of the electric current. On the other hand, sulphocyanide would have a small lyotropic effect, and there would be little diminution in resistance.

There are at least three serious objections to this as an explanation of the phenomena observed. In the first place, the relative powers of the various salts to cause precipitation of albumen and to cause a decrease of resistance of *Laminaria* are out of all proportion to their relative powers of attracting water, as measured by the amount of water taken up by the dry salt. Sodium sulphate takes up about twice as much water as sodium citrate, and yet its lyotropic effect is only about seven-tenths as much, according to HOFMEISTER'S experiments with egg albumen and the writer's with *Laminaria*. In the second place, there would not be so many variations of the series if it were dependent simply upon water attraction. POSTERNAK (**16**) showed that acidity influenced the series, while HÖBER (**4**) showed that concentration is also a factor. These so-called "transition series" demonstrate that the matter cannot be explained on a simple water attracting hypothesis. A third objection is that even if this theory accounted for the decreases in resistance found with salts like sodium chloride and sodium citrate, it could not account for the increases obtained with such salts as magnesium and calcium chlorides. Calcium chloride is surely more water attracting than sodium chloride, and upon this hypothesis it is difficult to see why one should cause a decrease and the other an increase in resistance.

A second explanation worthy of only scant attention is that of SCHADE (**22**). He conceives that under the influence of the neutral salts water is polymerized into particles or molecules of the structure $(H_2O)_n$. Salts like sulphate and citrate are able to produce large groups, while chlorides and nitrates produce smaller ones. Since these molecules may be conceived of as any size convenient for the

purpose, this theory avoids the first objection raised. It does have the other objections named, however, although SCHADE might possibly explain the rise in resistance of *Laminaria* produced by $CaCl_2$ by saying that the normal state of water was $(H_2O)_n$, as many have now come to think, and that the $CaCl_2$ causes the normal aggregates to split up so that the normal solubility of the substances composing the filter of the semipermeable membrane is increased, resulting in fewer pores than normally, with its attendant decrease in ionic conductivity. SPAETH (23) offers a simple viscosity explanation of the phenomena, but gives no explanation of the action of the coagulating agent, and does not explain adequately why such "coagulating" substances like $CaCl_2$ cause first a decrease and later an increase of permeability.

A third explanation, which borders close upon some of the others, is that of HÖBER, who tries to reconcile such diverse results as those obtained by HOFMEISTER and OVERTON (13), in which the salts fell into two groups of opposite behavior, the results found in the studies on the precipitation of gelatine, and other similar phenomena. It will be recalled that HOFMEISTER, working with a gel like gelatine, found that sulphate, tartrate, citrate, and acetate caused a shrinking, while chloride, bromide, and nitrate caused a swelling. In like manner OVERTON found that when frog's muscle was placed in potassium sulphate, tartrate, phosphate, and acetate from 24 to 50 hours and then placed into a pure solution of NaCl, it regained its irritability quickly, but when it had been placed in solutions of chloride, bromide, and nitrate it did not. HÖBER tries to reconcile these results with the HOFMEISTER series by suggesting that we have to do with two processes, one in which the anions work in the same way in varying degrees, and one in which some work one way and some another. For example, in the precipitation of egg albumen they all act the same way in varying degrees, while in the swelling of gelatine they act in two ways, one group causing a swelling, the other a shrinking. This does not seem to be very much of an explanation, but rather the restatement of an experimental fact.

A fourth theory is that of LOEB (6), who explains the results obtained by assuming ion-protein combinations. According to this conception the ions of the salts form combinations with the proteins

of the cell. These ion-protein compounds may influence permeability in various ways. In many cases they obey the mass law, indicating the formation of real chemical compounds. Those ions with the largest charges have the most influence, which explains why citrates and phosphates have a much greater "salting out" effect than monovalent anions like chlorides and nitrates. A more recent theory to explain the antagonism effects between sodium chloride and calcium chloride is that of OSTERHOUT (10), who explains this antagonism by assuming that the resistance to the electric current is produced by a compound formed by some substance in the protoplasm with the sodium and calcium chlorides. Calcium chloride favors the formation of this compound, while sodium does not. Throughout his work he assumes that antagonism is primarily between the cations.

The writer believes it is possible to construct a hypothesis on the basis of electrical charges which will explain and correlate more of the phenomena than the explanations previously offered, and which will certainly explain many of the phenomena which have been found and described in connection with the study of *Laminaria*. This hypothesis may be briefly outlined as follows. It is probable that most of the organic colloids of the plant and animal body which are bathed in the blood or in sea water are negative (in the sign of their electric charge). MINES (9) has shown that the blood corpuscles of *Scyllium* are negative. It is common knowledge that sea water is slightly alkaline, and PERRIN (15) has shown that the H-ion concentration of a solution, even when very dilute, has a powerful influence upon the sign of colloids suspended in it. This negative charge upon the semipermeable membrane of *Laminaria* is extremely important in explaining the salt effects.

In respect to the action of salts upon protoplasm, they may be divided into three groups depending upon the relation between the density of the charge upon their cations to that upon their anions, or upon the strengths of the surrounding magnetic fields. (1) In salts like sodium chloride, magnesium sulphate, and aluminum citrate the charge upon each cation is approximately the same in density as upon each anion. Both ions in each of these salts are of the same valency. (2) In salts like sodium sulphate, sodium

citrate, and magnesium citrate the anion has a charge of greater density than the cation, since the anions in these three salts have greater valencies than the cations. (3) In salts like calcium chloride, ferric chloride, and ferric sulphate the reverse is true, and there is a higher potential upon the cation than upon the anion. For evidence that such differences may exist among ions reference is made to the work of VON HEVESY (3).

The experiments conducted by OSTERHOUT (11) and by the writer (17) show that the first two classes of salts act in a similar manner (but differing in degree), while the third class acts in an opposite manner on the protoplasm. It is not difficult to imagine a set of conditions in the protoplasm which would account for this behavior of the salts. For example, if the plasma membrane is composed of negative particles (as previously discussed) arranged in a network, and the ions combine with these particles, making some particles more positive and others more negative, it is easy to see that the electrical forces involved will cause the meshes of the network to expand or contract. The first two classes of salts would cause the meshes to expand, and for convenience may be designated as negative salts. The third class would cause the opposite effect, and may be designated as positive salts. Strong acids act like positive and alkalis like negative salts because the hydrogen and hydroxyl ions are extremely active.[1]

With slight modifications this hypothesis may be applied equally well if we suppose the plasma membrane composed of particles in suspension which are not united into a network.

[1] If a picture of just how this might happen is demanded, the following is suggested. Suppose a salt of the first category is used, such as NaCl. If to a given protoplasmic surface a molecule of NaCl is added, the plus charge of the sodium will neutralize a negative protoplasmic unit charge, leaving the same number of negative charges as before. The substituted Cl unit, however, being smaller than the protoplasmic one, has a more highly concentrated surface charge, and the result will be a readjustment of the relative positions at a little greater distance than formerly. The effect is a "loosening up" of the mesh or an increase in permeability. No matter how much NaCl is added, negative units will always remain in excess. When all the protoplasmic charges are neutralized, the permeability is at its maximum. If a salt of the second category (such as Na_2SO_4) is used, much the same effect is observed, but in an increased degree. An additional factor must here be considered, since there is a great difference between the concentration of the charges on the cation and the anion. The anion with two charges is much more effective than two cations with one charge each. Hence the

The essential points in this hypothesis may be summed up as follows: (1) The normal charge upon the particles comprising the plasma membrane of the cell is negative. (2) Salts may be classified in respect to their action upon the plasma membrane as positive and negative. Negative salts (and alkalis) cause the particles of the membrane to separate, thus decreasing electrical resistance and increasing permeability. Positive salts (and acids) have the opposite effect during the first few minutes of exposure, but later produce effects similar to those of negative salts. (3) Antagonism exists between salts which produce dissimilar electrical effects on the protoplasm.

It will be advantageous at this point to see how this hypothesis explains many of the phenomena in the literature cited, and to discuss the published results of the writer from this point of view. The work of HOFMEISTER shows the importance of valency and electrical charges very decidedly. Even if the water attracting power of a substance plays a part, the recent work of LOEB (7) on the electrification of water shows that water attraction itself is closely connected with electric charges and valency. HOFMEISTER's work on the swelling of gelatine, in which he found that the neutral salts were divided into two groups, has also been connected with electrical charges by LOEB (8), who finds that the swelling of gelatine and other physical properties connected with it depend almost entirely upon the charge present upon it.

These studies on the transition series are very interesting, viewed in the light of what has been said. From the writer's hypothesis we should expect to get in acid solutions results which are just the reverse of those obtained in alkaline. HÖBER's studies upon the

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salt acts as a very powerful "negative" salt. In like fashion sodium citrate would be much more "negative," which is exactly what is found. A salt of the third series acts oppositely to the former. Here the cation bears the more concentrated charge and is hence the determining factor in the behavior of the salt. The first effect when $CaCl_2$ is added to *Laminaria* tissue is the neutralizing of the negative units by the Ca charges. The meshes of the protoplasmic net are tightened and the structure is less permeable. Shortly all the negative charges are neutralized and replaced by positive ones when repulsion sets in and the surface becomes more permeable. The effect of a specific charge will depend upon the size of the ion which bears it and its speed. For this reason acids with the extremely active H-ion behave like positive salts, and alkalis with their active hydroxyl ions behave like negative salts.

concentration are of special interest. It is not difficult to understand that the ratio between the number of charges added by the solution and the number of particles present in the colloidal surface of the protoplasm would be very important. Most of the work in which permeability plays a rôle can be explained on the same basis as the work on *Laminaria*.

Antagonism under this hypothesis is the natural thing to expect between positive and negative salts. In mixtures there would result a diminution of the injurious effect of each, since each salt tends to disturb the equilibrium in the opposite direction. Calcium chloride, a positive salt, is hence antagonized by sodium chloride, which is a negative salt. It is merely the antagonism between a salt which tends to decrease the permeability of the plasma membrane and the one which tends to increase it, due to the negative nature of the membrane itself and the relation which exists between the charges on the effective ions. As OSTERHOUT has pointed out (10), any theory which accounts for the permeability effects must explain: (1) why both sodium chloride and calcium chloride are toxic; (2) why when mixed in proper proportions their toxicity is diminished; (3) why one causes an increase and the other a decrease in resistance; (4) why the decrease caused by calcium chloride is later followed by an increase; and (5) why toxicity disappears in sea water.

The preceding hypothesis explains all of these matters. Sodium chloride and calcium chloride are toxic because they change the electrical condition of the permeable surface. The one makes the surface more permeable, the other less. When mixed in proper proportions, the toxicity diminishes because of the neutralizing effects of the dominant electrical charges carried by each salt. NaCl causes an increase in permeability because it is a negative salt, due to the fact that the protoplasm is negatively charged. $CaCl_2$ causes an increase in resistance because it is positive salt, hence its first effect is to cause the units of the permeable surface to approach each other, and the permeability is hence decreased. As the negative charges are later replaced by those carried by the dominant action of the cation of the salt, the units again repel each other and the permeability is increased.

In sea water toxicity disappears because of the balance existing between the various ions which compose it. It is interesting that of the six normal plant elements taken in quantity from the soil three occur in the cation with five positive charges, while three occur in the anion with six negative charges (K^+, Ca^{++}, Mg^{++}–N^-, S^{--}, P^{---}). This is too nearly balanced to be a mere coincidence. Furthermore, this hypothesis explains why $MgCl_2$, $AlCl_3$, etc., cause one type of permeability curve, and NaCl, $MgSO_4$, etc., another. It also shows why acids with an extremely active ion bearing a concentrated positive charge behave like $CaCl_2$, and why alkalis behave like NaCl.

The general effect of salts may be predicted, and the following generalization made. Salt solutions of approximately the same osmotic pressure, conductivity, and acidity as sea water will cause an initial increase in resistance of *Laminaria*, followed by a fall if the valency of the cation is greater than the valency of the anion, and will cause a continued decrease in resistance of the tissue from the start if the valency of the cation is equal to or less than that of the anion.

An interesting apparent exception is the antagonism reported by OSTERHOUT (12) between sodium chloride and sodium taurocholate. While these salts are in the same class with respect to valency, the former acts like a negative and the latter like a positive salt. They antagonize each other as is to be expected, but the question is, Why is sodium taurocholate a positive salt? The answer is probably to be found in the large size of the anion, which makes its surface density relatively small as compared with the sodium. The result is that the positive charge has more effect upon the plasma membrane than the negative charge on the taurocholate, and the salt is positive. The formula of this salt is $NaC_{26}H_{44}O_7NS$, making a cation of atomic weight 23 and an anion of 514. The concentration of the charge is so much greater on the cation than on the anion that the cation is dominant, and the salt is positive like $CaCl_2$.

The hypothesis outlined here unifies most of the writer's published work in this field. It explains why the anions both inorganic and organic fall into three groups, depending on their valency

(17, 18), why a mixture of aluminum citrate and aluminum chloride, or of magnesium citrate and magnesium chloride produces less increase in resistance than $AlCl_3$ or $MgCl_2$ alone (19), why $CrCl_3$ causes an increase in resistance and Na_2CrO_4 a decrease, the one being a positive and the other a negative salt (20), and finally why $FeCl_3$ causes a greater increase in resistance than $FeCl_2$ (21). In justice it should be admitted that the antagonism between sodium acetate and sodium sulphate, both negative salts, or between $CaCl_2$ and $MgCl_2$, both positive salts, is not so clearly explained. These cases are exceptions, however, and other factors beside electrical charges doubtless enter in. That electrical phenomena play a part even in the miscellaneous assortment of cases reported is evinced by the fact that most of these reported cases of antagonism between anions are between anions of different valency. It should be pointed out also that the availability of the charge must also be considered, and that, with such complex situations as the cell surface presents, where many factors may enter in, it will doubtless be some time before all the phenomena are correlated. This is merely an attempt to explain more of the permeability phenomena by a single explanation than have thus far been explained by any one contribution. It may thus be considered a step in the right direction. It is perhaps too early to hope for the master key, which will open all doors at once, and it may develop that these phenomena have no common explanation, but belong in entirely different categories.

It should be mentioned that this hypothesis does not disagree with the protein theory of the composition of the cell surface. Since salts have similar effects on proteins, as on the cell, it is only natural to suppose that the same substances are concerned in both cases. There is certainly nothing in this work which confirms the lipoid theory of the cell surface, although the hypothesis does afford a very interesting explanation of OVERTON'S work (14). As BAYLISS (1) points out, OVERTON'S dyes not only were divided into two groups on the basis of their lipoid solubility, but also in their composition. The dyes which penetrated the cell were the so-called "basic" dyes, made of a color base and some ordinary acid such as

HCl, with the formula BaseCl, while those which did not penetrate were the "acid" dyes, made of a common base such as Na and a color acid with the formula NaAcid. Since in the basic dye the cation is much larger than the anion, the concentration of the charge is much greater on the anion, and the dyes are hence negative in the terms of this hypothesis. Under their influence, as under that of Na_2SO_4, the permeability of the cell is increased and the dye readily enters.

Summary

The effects of a salt upon the permeability of *Laminaria* may be predicted according to the following rule. Neutral salt solutions of the same osmotic pressure and conductivity as sea water cause an initial decrease in permeability, followed by an increase if the valency of the cation is greater than that of the anion. They cause an increase in the permeability from the start if the valency of the cation is less than that of the anion. If the two are of the same valency the effect will depend upon the relative size of the ions and the density of the accompanying electric charges.

A hypothesis is proposed to explain the observed phenomena which may be summarized as follows: (1) Permeability is due to the electrical condition of the semipermeable membrane, which in *Laminaria* is negatively charged when in sea water. (2) A solution in which the cation carries the more dense charge, a "positive" salt, alters the charge on the membrane and decreases the permeability. As more of the salt diffuses in, the negative charges on the membrane are replaced by positive charges, the effect is reversed, and the permeability increases. Acids are "positive" electrolytes. (3) A solution of a salt in which the anion carries the more dense charge, or in which the charges are approximately equal on the two ions, a "negative" salt, increases the negative charge on the membrane and increases the permeability, which continues until the death of the tissue. (4) Antagonism exists between those salts which produce dissimilar electrical effects upon the protoplasm.

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ACTION OF ULTRAVIOLET LIGHT ON YEAST-LIKE FUNGI. II

FRED W. TANNER AND EARL RYDER

In August 1920 there was published from this laboratory a paper (9) dealing with the action of ultraviolet light on yeast-like fungi. It was shown that yeast cells are little more resistant to the action of this form of energy than other forms of life. Under the conditions of the experiment described in that paper, ten minutes was the maximum that yeast cells could endure the action of ultraviolet light. BUCHTA (2) had previously reported three minutes as the limit of endurance. In spite of these papers dealing directly with yeasts, and the many other published investigations stating that bacteria, protozoa, and even higher plants are quite sensitive to these rays, FAZI (8) challenges the results from our laboratory, as well as those from other laboratories, as follows:

> In my experiments I exposed brewer's yeast for 12 hours to the ultraviolet rays from a 1200 candle power lamp at a distance of 20 cm. Not only was the yeast not injured by this treatment, but its fermentative activity was actually increased. All of the bacteria in the yeast were destroyed after a brief exposure.

Such a conclusion seems unreasonable in light of the known toxicity of this light to practically all forms of life. This project was continued in these laboratories in order to satisfy ourselves more thoroughly with regard to the validity of our former data, and also, if possible, to find the reasons for the discrepancies in our data and those of FAZI. The method of FAZI was to expose the cells in dextrose and water to the action of the rays emitted from a 1200 candle power lamp operating at 110 volts and 4 amperes, at a distance of 20 cm., and test the fermenting ability of the cells by measuring the amount of carbon dioxide formed. He reported that a suspension of yeast cells, after exposure to the ultraviolet radiations, caused an acceleration of the fermentation, as was noticed when the fermenting solutions themselves were exposed. A solution of dextrose inoculated with beer yeast at 20 cm. from a 1200 candle power Cooper-Hewitt lamp formed about twice as much carbon dioxide as a similar solution not so exposed.

Experimental work on the effect of ultraviolet rays on microorganisms is too voluminous to review completely at this time, but some of the more important contributions may be mentioned. MAURAIN and WARCOLLIER (13) found that ultraviolet radiations would completely arrest fermentation in cider, but that the cider had to be exposed to the radiations in a very thin layer. The acetic acid fermentation of wine was also found to be inhibited by SCHNITZLER and HENRI (14). The conclusions of MAURAIN and WARCOLLIER that very thin layers must be used for exposure may explain why FAZI noticed no inhibition of fermentation, but not the statement that cells so treated were absolutely unharmed and exhibited greater fermentative activity.

Bacteria are also quickly destroyed by ultraviolet light. CERNOVODEANU and HENRI (4, 5) reported a destruction of bacteria which differed with the organisms. The rate was almost proportional to the square of the duration of exposure. In 1910 these same investigators studied the protoplasmic changes induced in cells of bacteria, yeasts, trypanosomes, paramecia, and blood cells. The protoplasm in these cells was shown to be distinctly changed. For instance, Gram positive bacteria were rendered distinctly Gram negative, and acid fast bacteria lost their acid fastness. The time required to destroy the cells was not given. GARTNER is said to have observed the same phenomena in bacteria exposed to these rays (BUJWID 3). BUJWID studied the use of ultraviolet rays in water treatment, and reported that pathogenic bacteria were destroyed in a few seconds. LEMATTE (12) and LANZILLOTTA (11) have stated that bacteria killed by ultraviolet light are suitable for vaccines. The latter worker found that an exposure of 20 minutes killed the cells, but did not destroy their antigenic properties. FAIRHALL and BATES (7) studied the use of ultraviolet light on bacteria suspended in oil. An exposure of 3–5 minutes at a distance of 10 cm. was sufficient to kill the bacteria. Their object was to test the use of this form of energy in the preparation of lipobacterins. A similar study was carried out by EBERSON (6), who exposed salt suspensions containing 2000 million meningococci per cubic centimeter to ultraviolet rays. Heat effects were ruled out by working at distances of 36 inches. Growth of the organisms was completely inhibited in 7

minutes for the para, 7.5 minutes for the regular, and 10 minutes for the irregular strains. This time of survival was somewhat longer than that reported by other investigators, due probably to the use of very heavy suspensions of cells. As CERNOVODEANU and HENRI, and GARTNER have shown, EBERSON found a marked change in bacterial protoplasm. This was measured by agglutination reactions. As would be expected, a variation in resistance between vegetative and spore cells has been observed by LAGERBERG (10). Spores of *Bacillus anthracis* were much more resistant than vegetative cells, but spores of *B. subtilis*, *B. megatherium*, and *B. mesentericus* seemed to have the same resistance.

Several investigations have been reported on the resistance of enzymes and antibodies to ultraviolet rays. A paper bearing directly on the subject was published by BURGE, whose object was to determine whether intracellular enzymes of bacteria are destroyed when the bacteria are destroyed by ultraviolet light. He used cultures of common bacteria, such as *Erythrobacillus prodigiosus*, *Pseudomonas fluorescens*, *P. pyocyanea*, *Proteus vulgaris*, and *Bacillus subtilis*. These were taken because they liquified gelatin. The bacteria were exposed until dead, after which they were ground up in sand and 30 per cent alcohol, to extract the enzymes, and 10 cc. of this alcoholic extract added to tubes of sterile gelatin. Only slight differences were observed between the amount of gelatin liquified by the living bacteria and by their extracted enzymes, indicating that the endoproteases survived exposure to the ultraviolet rays. They may have been protected from the effects of the rays by coagulated protein about the outside of the cell, but this would be true with any cell. In another paper BURGE showed that egg white was coagulated, and argues that coagulation must be the explanation of the abiotic effects of ultraviolet, and not that the endo-enzymes have been destroyed.

This brief survey of the literature indicates several things. What work has been published on the action of ultraviolet light on yeasts indicates that they do not possess any marked resistance to it. They seem to be only slightly more resistant than bacteria. Exposure of fermenting solutions to the action of the ultraviolet light inhibits fermentation. The fermenting ability of yeast

does not seem to be greatly increased by exposure to ultraviolet light.

Experimental work

The apparatus used in this experiment was a 1200 candle power Cooper-Hewitt lamp operated at 110 volts and 4 amperes (D.C.) at a distance of 20 cm. There was no appreciable rise in temperature at this distance. These conditions were maintained constant during all the experiments except where statements to the contrary appear. The following yeasts were used: (1) *Saccharomyces ellipsoideus* Hansen; (2) yeast from oyster (Hunter); (3) *Saccharomyces anomalus* Hansen; (4) *Torula datilla;* (5) *Endomyces albicans* Vuillemin; (6) *Saccharomyces glutinis* Cohn; (7) *S. albus;* (8) *S. marxianus;* (9) *S.* of Curtis; (10) yeast from vinegar; (11) *Cryptococcus glabratus;* (12) *Saccharomyces hominis* Busse; (13) Burgundy wine yeast; (14) *Cryptococcus Ludwigii;* (15) *Torula communis* Browne; (16) *Cryptococcus agregatus* Anderson; (17) champagne yeast; (18) *Zygosaccharomyces bisporus* Anderson; (19) *Saccharomyces cerevisiae* Hansen; (20) *Torula humicola* Daczewska. These strains have been in the laboratory collection under these names for some time. Before use they were carefully purified.

A platinum loop of each yeast was suspended in 100 cc. of sterile water. This suspension was then gently agitated on a shaking machine for ten minutes in order to insure the breaking up of any clumps of cells. One cc. of the suspension was then placed into 99 cc. of sterile water, giving a 1–100 dilution. It was found that, in the majority of cases, this dilution produced a concentration of colonies on the Petri dishes that permitted accurate counting. The diluted yeast suspension was then poured into a covered Petri dish, and placed directly under the ultraviolet lamp, the thickness of the liquid layer being about 8 mm. Just before the experiment was started the cover was removed from the dish. A control plate was made by taking 1 cc. of the unexposed suspension and plating it out in acid Saboraud's agar. The current was then turned on and 1 cc. portions of the suspension were removed from the Petri dish under the lamp by means of sterile pipettes and put into sterile Petri dishes. At definite intervals the number of living bacteria was determined. Saboraud's agar was used in pouring the plates. Before pouring, 4 cc. of 5 per cent lactic acid was

added to each 100 cc. of the melted agar. This acid concentration was found to allow rapid growth of the yeast colonies, at the same time preventing the appearance on the plates of colonies of bacteria if they happened to get in. Throughout the entire time of exposure the suspension in the dish was stirred gently in order to insure equal exposure of all parts of the liquid to the rays.

The first experiment was carried out to determine the length of time that the yeast cells could survive continuous exposure under these conditions, and to determine the rate of death. Suspensions

TABLE I

RATE OF DEATH OF PURE CULTURES OF YEASTS EXPOSED TO ULTRAVIOLET LIGHT AT DISTANCE OF 20 CM. (DILUTION 1–100)

Name	No. of cells at beginning	Minutes required for destruction
Saccharomyces ellipsoideus	140,000	7
Yeast from oysters (Hunter)	271,000	20
Saccharomyces anomalus	20,500	14
Torula datilla	215,000	5
Endomyces albicans	10,500	2
Saccharomyces glutinis	37,900	10
Saccharomyces albus	81,300	5
Saccharomyces marxianus	29,800	6
Saccharomyces of Curtis	5,200	8
Vinegar yeast	456,300	4
Cryptococcus glabratus	330,700	5·5
Saccharomyces hominis	26,400	1·5
Burgundy wine yeast	76,300	2
Cryptococcus Ludwigii	5,472,000	7·5
Torula communis	55,600	5·5
Cryptococcus agregatus	113,200	7·5
Champagne yeast	114,400	1·5
Zygosaccharomyces bisporus	76,800	5
Saccharomyces cerevisiae	256,000	9
Torula humicola	82,500	7·5

of yeast cells were prepared by putting a loop of growth from an agar slant into a small amount of water and shaking on a shaking machine for about ten minutes. This homogeneous suspension was then poured into sterile Petri dishes, the depth of the layer of yeast suspension not being more than 1 cm. The dish was then placed under the lamp with nothing intervening between the lamp and the liquid containing the suspended cells, so that the cells were exposed directly to the ultraviolet rays. Some of the results for the yeasts used are shown in table I. These data show that under the conditions of the experiment the yeasts exhibited no such remarkable

resistance to ultraviolet light as was reported by FAZI. In one case (culture no. 2, yeast from oysters, Hunter) there was a survival period of eighteen minutes, about twice as long as observed with the other cultures. This is explained by the fact that this variety is a deeply red pigmented form. According to the observations of many investigators, ultraviolet rays are unable to penetrate a colored solution as rapidly as a colorless one. Consequently, it could not penetrate the pigmented cells of this yeast as rapidly as the colorless cells, and a longer time was required for killing. VALLET (15) has discussed the importance of the physical and chemical characteristics of the medium exposed to the action of ultraviolet rays.

In order to secure some data on bacteria with which the data on the yeasts could be compared, the death rate of eight common bacteria was studied. FAZI stated that the increased fermenting ability of yeast which had been exposed to ultraviolet rays was due to destruction of contaminating bacteria. The results for five of these cultures are shown in table II. It will be seen that there was

TABLE II

RATE OF DEATH OF PURE CULTURES OF BACTERIA WHEN EXPOSED TO ULTRAVIOLET LIGHT

Name	No. of cells at beginning	Time required for killing
Bacterium coli	258,900	3.5
Bacillus cereus	177,600	6.0
Bacillus capsulatus	684,000	4.5
Bacillus cyaneus	979,000	3.0
Bacterium aerogenes	2,822,400	4.5
Bacillus roseus	About 200,000	1.5
Bacillus subtilis	About 244,000	3.5
Bacillus cloacae	972,800	3.5

a rapid death rate not much different from that for most of the yeasts. From these data it does not seem that bacteria in a mixture of bacteria and yeasts would be destroyed in a markedly shorter time than yeasts. It is probable that, generally speaking, a more rapid death rate would exist for bacteria than for yeasts, since the bacterial cell is so much smaller, and the toxic ultraviolet rays would reach the vital parts of the bacterial cell more quickly than in the yeast cell.

Since it has been claimed by FAZI that ultraviolet light accelerates fermentation by destroying contaminating bacteria, several experiments were carried out with yeast suspensions purposely contaminated. Yeast suspensions of *Saccharomyces ellipsoideus* were prepared by shaking two loops of a 24-hour culture in 100 cc. of sterile water. These suspensions were then contaminated by adding 5 cc. of soil suspension. Five cc. of this suspension were introduced into the control flask, and the remainder of the suspension exposed to the radiations from the ultraviolet lamp in the same manner as previously mentioned. At definite intervals of time, 5 cc. of the suspensions were withdrawn from the dish in which it was exposed and inoculated into one of the fermentation flasks fitted with the ALWOOD (1) ventilation valve containing sulphuric acid. The stoppers were tightly turned into the necks and the flasks immediately weighed. They were allowed to ferment for thirty days, during which time the loss of weight was determined. The results are shown in the diagram (p. 316).

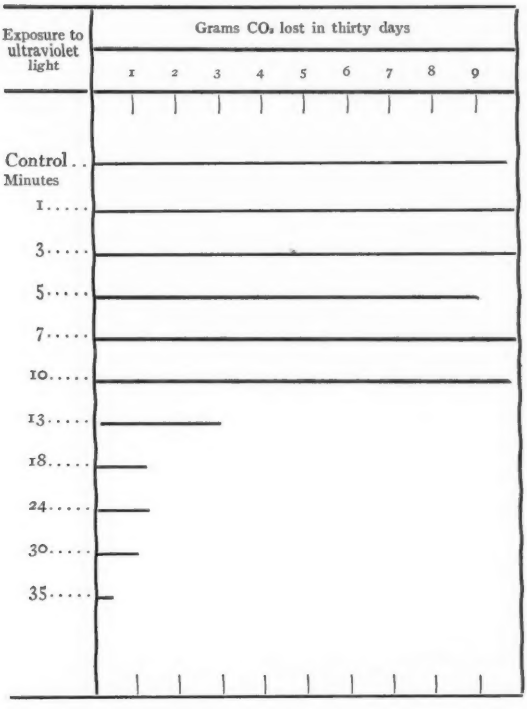
This experiment was repeated with a yeast suspension contaminated with sour milk and sewage. With such mixtures the yeasts would be involved in a greater diversity of bacterial types than a culture used by a fermentologist. Since these results are not significantly different, only the data for the soil suspension yeast experiment are given.

These data do not indicate that exposure to ultraviolet light causes a marked increase in the activity of yeasts, nor that yeasts are able to form much larger amounts of carbon dioxide after exposure to ultraviolet radiations, nor that they are able to withstand prolonged exposure. In the yeast suspensions mixed with soil extract and sewage a marked decrease in the amount of carbon dioxide is secured after exposure to ultraviolet rays for 5–7 minutes.

To test the validity of the claim that exposure of yeast to ultraviolet rays increases its fermenting power on account of the destruction of contaminating bacteria, mixtures of pure cultures of bacteria and yeasts were exposed to the rays and counted at frequent intervals. It was found that bacteria dropped out in a slightly shorter time than yeasts. In each case they were followed so quickly by the yeasts, however, that the difference in time was of

little significance. HENRI, HELBRONNER, and RECKLING-HAUSEN report similar results for artificial mixtures of *Sarcina* and yeasts.

EFFECT OF VARIOUS EXPOSURES TO ULTRAVIOLET LIGHT ON FERMENTING ABILITY OF YEAST SUSPENSIONS CONTAMINATED WITH SOIL EXTRACT



Summary

1. No evidence was secured to show that yeast cells possess any marked resistance to ultraviolet light. They were able to live only from a few seconds to a few minutes longer than bacteria. This is reasonably explained by the difference in size.

2. Pigmented yeasts are more resistant than white yeasts. This is due to the resistance to penetration of the rays.

3. Exposure of contaminated yeast cultures to ultraviolet rays did not increase their fermenting abilities. This is in accord with

the data of MAURAIN and WARCOLLIER for cider fermentation, and SCHNITZLER and HENRI for acetic acid fermentation.

4. The data presented herewith and that of other investigators indicate that there is a direct relation between the size of the cell and its resistance to ultraviolet light.

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BRIEFER ARTICLES

AN OLD BRITISH HERBAL

A copy of an old British herbal,[1] written by a contemporary of LINNAEUS, is in the hands of a book dealer[2] in Victoria, British Columbia. The frontispiece represents, according to its legend, "The Genius of Health receiving the tributes of Europe, Asia, Africa, and America, and delivering them to the British Reader." The Genius holds a scroll reading "The British Herbal 1756." A woodcut on the title page represents "Aesculapius and Flora gathering from the Lap of Nature, Health and Pleasure." The volume is dedicated "To the Right Honorable The Earl of Northumberland," who is addressed as "Patron of Useful Knowledge" and "Father of the Distressed," allusion being made to the founding of a hospital by him.

Each plate shows many plants. The figures show accurately the more obvious parts of the plants, but no details of parts. They are what might be called "habit sketches." The use of terms in this volume illustrates the state of botanical knowledge at the time. The terms "threads" and "filaments" are used for what we now call stamens, and "a single petal" is used to designate what was later called a gamopetalous corolla.

More than twenty authors are quoted, although the name of the volume referred to is rarely given, and page citations are extremely rare. C. BAUHINE and J. BAUHINE are referred to most frequently, although references to LINNAEUS, TOURNEFORT, and RAY are common. In most cases a reference consists merely in a statement of the name by which the author referred to called the plant under discussion. In a few cases, however, the writer discusses the classification used by the person whose work is referred to. In this regard he often finds occasion to criticize LINNAEUS, although occasionally he breaks the monotony by commending him for something. He speaks with great respect of RAY, frequently

[1] The British Herbal: an history of plants and trees, natives of Britain, cultivated for use or raised for beauty. By JOHN HILL, M.D. London: Printed for T. OSBORNE and J. SHIPTON, in Grays Inn; J. HODGES, near London Bridge; J. NEWBERRY, in St. Paul's Churchyard; B. COLLINS; and S. CROWDER and H. WOODGATE, in Paternoster Row. pp. iii+533. index. *pls.* 75. MDCCLVI.

[2] This volume is now owned by the University of Washington.

writing "Mr." RAY and speaking of him as one "who followed nature carefully." Among the other authors mentioned are DILLENIUS, PLUKENET, MORISON, MENTZELIUS, COMMELIN, PARKINSON, ALPINUS, HERMAN, and PETIVER.

The whole plant kingdom is divided into 35 classes, the distinctions between classes being based mostly on the nature of the flower. Class 33, however, is "Trees and shrubs." Class 34 is "Plants whose flowers and seeds are minute and singly inconspicuous; and are produced on the backs of their leaves." Under this head are put *Polypodium*, *Asplenium*, *Adiantum*, and other common ferns. Class 35 is "Plants whose flowers and seeds are minute and singly inconspicuous and are not placed on the back of the leaves." A partial list of the plants of this class is *Ophioglossum*, *Equisetum*, *Chara*, *Conferva*, *Ulva*, *Mnium*, *Fontinalis*, *Hypnum*, *Polytrichum*, *Bryum*, *Sphagnum*, *Lycopodium*, *Lichen*, *Fungus*, and "submarine plants," the last being described as "vegetables growing under sea water, with minute and uncertain fructifications." The genera mentioned under submarine plants are *Corallina*, *Fucus*, and *Alga*, the figure under "Alga" being apparently *Zostera*.

The Latin names for plants are in some cases binomials, although in a few cases the name consists of a single word, in many cases of three or four words, and in some cases of as many as six words. The binomials may be illustrated by *Argentina vulgaris*, *Caltha palustris*, and *Aquilegia sylvestris*, while examples of the monomials are *Hottonia*, *Chamaemorus*, *Samolus*, and *Pulsatilla*. Examples of longer names are *Chara cinerea fragilis*, *Lichen foliis latioribus*, *Fontinalis major foliis trianguloribus*, *Caryophyllata foliis incisis caule repente*, and *Aquilegia magno flore foliis majus divisis*.

The uses mentioned for plants are largely medicinal. The following illustrations are taken at random. Dodder, buckthorn shrub, and the bindweeds are mentioned as purges, while cinquefoil is "recommended to stop purgings." Herb Robert is said to be astringent, and common tormentill is stated to be "good in haemorrhages." White dittany, sweet beard, and saffron are cordial and sudorific. Blue hepatica is vulnerary. Spanish verona is "good in sciatica," and pilewort "does great service in scrofulous tumors." Larkspur is said to be "good against disorders of the eyes," while perennial-rooted adonis and the crowfoots are stated to "have juice that will drive away warts." Herb mastic is spoken of as "a warm aromatic plant" that is "good in nervous disorders." Asparagus is recommended as being "excellent against gravel." Catmint tops beat into a conserve are said to be "good against that

troublesome and obstinate disorder called the nightmare," and dovesfoot is "recommended against rupture." Among the plants mentioned as poisons are monkshood and hemlock. Among the plants used for human food are the rock rampion, which "is eaten in the manner of radishes," and the dandelion, which is used for salads. Flowers of meadowsweet are said to "give a pleasant flavor of liquor." Water gladiole is said to be "food for ducks and other waterfowl."

The following facts about HILL are taken from the extended biography of him given by LEE.[3] Besides *The British Herbal*, he published other herbals, as well as some more extended and specialized works on the treatment of diseases by means of herbs. His writings on plants include some works on practical gardening, as well as such titles as the sleep of plants, the cause of motion in the sensitive plant, and a method of producing double flowers from single ones. His botanical works did good service in their day, and the first Linnaean flora of Great Britain was due to him. He was an apothecary and later a quack doctor. He died of gout, a disease for which he claimed to have a specific. He published many books and pamphlets on subjects outside of botany, most of which were controversial and trashy. Having failed to get into the Royal Society, he attacked it in satirical pamphlets. He also mixed a great deal in matters pertaining to literature and the stage, and had a paper warfare with FIELDING, abused CHRISTOPHER SMART, was hissed off the stage when attacking GARRICK, and was publicly thrashed by an Irishman named BROWN.—GEORGE B. RIGG, *University of Washington, Seattle, Wash.*

FURTHER NOTES ON EFFECT OF EXTENT OF ROOT SYSTEMS ON TILLERING OF WHEAT

In a previous paper,[4] the writer has shown that the extent of root systems of wheat plants when placed in good nutrient solutions plays no inconsiderable rôle in the amount of tillering that may be induced in the subsequent growth of the plants. As the cultures used in those experiments were seedlings of different ages, it was deemed well to conduct further tests with plants of the same age, having also marked differences in extent of root growth. To obtain such cultures, wheat seedlings 6–8 cm. high were supported on corks fitted into Mason jars filled with

[3] Dictionary of National Biography **26**: 297.

[4] GERICKE, W. F., Certain relations between root development and tillering of wheat; its significance in the production of high protein wheat. Amer. Jour. Bot. **9**:366–369. 1922.

tap water, and allowed to grow for six weeks. At the end of this time the cultures had a root mass measuring 50–60 cm. long,[5] which contained about one-half of the total dry weight of the plants. These cultures were then treated as follows:

TABLE I

NUMBER OF TILLERS OBTAINED FROM CULTURES OF FIVE PLANTS EACH HAVING DIFFERENCES IN EXTENT OF ROOT SYSTEMS WHEN PLACED IN GOOD NUTRIENT SOLUTION

Set I (roots 50–60 cm. long)	Set II (roots 30 cm. long)	Set III (roots 4 cm. long)
20	17	10
17	19	11
23	19	10
23	18	13
20	15	10
16	17	12
19	15	11
22	14	13
Average per culture....20	16.7	11.2

SET I.—Eight cultures without any further treatment were transferred in a good nutrient solution.

SET II.—Eight cultures had their roots cut off 30 cm. from the root crown, and were then placed in the nutrient solution.

SET III.—Eight cultures had their roots cut off 4 cm. from the root crown, and were then placed in the nutrient solution.

The nutrient solution used for the three sets was of the same composition and concentration, the latter being approximately one atmosphere osmotic value. It consisted of equal partial molecular concentrations of KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$. A trace of $FeSO_4$ was added to each culture at weekly intervals, and distilled water was used to replenish the loss occasioned by transpiration of the plants. The cultures were allowed to grow six weeks, when the amount of tillering (in numbers) was determined. The experiment was conducted in the greenhouse in the spring of the year, when growing conditions are very good.

Table I shows that the cultures having the largest root systems when placed in the nutrient solution produced significantly more tillers than did those having smaller root systems. While it may be argued that the cultures with the smaller root systems were injured by the removal of

[5] ———, Root development of wheat seedlings. BOT. GAZ. 72:404–406. 1921.

part of their roots, it should be appreciated that both sets II and III had practically the same number of roots cut, which implies that these two sets of cultures sustained the same degree of injury. Since the cultures of set II gave decidedly more tillers than did those of set III, the differences in amount of tillering obtained can therefore be assumed to be due to the extent of root systems these two sets of cultures had when the tests were started. This resulted in a difference in absorbing capacity for nutrients for the different sets of cultures, and seems to be a plausible explanation for the results obtained in the tests.—W. F. GERICKE, *Laboratory of Plant Nutrition, University of California, Berkeley, Cal.*

CURRENT LITERATURE

BOOK REVIEWS

Carotinoids and related pigments

Our knowledge of the carotinoid pigments which occur in plants and animals has been brought together in the form of a monograph by PALMER,[1] who has contributed much to this field during the last ten years. The first five chapters deal with the distribution of the pigments in nature by groups, the phanerogams, cryptogams, vertebrates, and invertebrates receiving attention in separate chapters. More than half of the text concerns itself with the data on distribution. The following chapters deal with the chemical and biological relations between the plant and animal carotinoids, methods of isolation, identification, and quantitative estimation of these pigments. The final chapter considers the possible functions of these pigments, and shows how little is known as yet about their functions, if they have any. Students of the pigments will find the book very useful, for it lists more than 450 titles, and PALMER has taken occasion to point out many of the gaps in our knowledge that future investigation should fill in. This feature should make it stimulating to those who are interested in this field.

The mechanical composition of the book is good, and does credit to the American Chemical Society Monograph Series of which it is a part. There is one unfortunate feature, however, which should have been avoided. There are a considerable number of misspelled scientific names in the parts of the books dealing with the distribution of the carotinoids. The reviewer found more than twenty such errors in the chapters on plant carotinoids. In addition, there are a few words in the text that were overlooked in the proof reading, and an inadvertent reference to Miss TAMMES as "he." These errors will not impair the usefulness of the book, which is a good summary of the work done on these pigments to the present time.—C. A. SHULL.

Chemistry of plant products

The third edition of this favorably known work[2] has been prepared in two volumes, in order to treat more fully the physiological aspects of plant chemistry without unduly increasing the size of the individual volume. The

[1] PALMER, LEROY S., Carotinoids and related pigments. 8vo. pp. 316. New York: Chemical Catalog Co. 1922.

[2] HAAS, P., and HILL, T. G., An introduction to the chemistry of plant products. 2 vols. 8vo. vol. I, pp. xiii+414. 1921; vol. II, pp. viii+140. Longmans, Green, and Co. 1922.

first volume covers the same general ground as the previous edition, with some minor rearrangements. A section on the aldehydes has been inserted between the fats and carbohydrates, and the chapter on the colloidal state has been somewhat expanded to give more adequate treatment.

The second volume deals with the metabolic processes of the plant, and presents some of the material formerly included in the physiological discussions of the original volume, with more extensive consideration. There are six chapters in this new volume, an introductory chapter, and chapters treating the synthesis of fats, carbohydrates, proteins, respiration, and growth. The authors have chosen to present a rather brief summary of metabolism suitable as an introduction to further study, rather than an exhaustive digest of the literature. All details of experimental methods have been excluded as belonging rather to a practical treatise. The second volume does not seem to the reviewer to maintain quite the same standard of excellence that marked the first volume and the preceding editions of this important work. There is a certain amount of loss in convenience in having the physiological discussions in a separate volume, although the first volume still retains part of the physiological treatment, particularly the significance of the various plant products in the physiology of the plant. On the whole the new edition is hardly to be considered an improvement over the second edition, except in so far as some of the later literature has been brought into the discussion.—C. A. SHULL.

MINOR NOTICES

First book of grasses.—Students of this interesting group of plants, and teachers of elementary classes in taxonomy and agronomy, should find much assistance and interest in the little volume written by Miss CHASE.[3] The simplicity of the beginnings and the graded character of the successive chapters are ideal for a class textbook, while the abundance and quality of the illustrations will do much to remove the difficulties usually encountered by beginners. Miss CHASE has evidently made a contribution likely to result in increased knowledge of a group usually regarded as difficult, and it is safe to predict that her efforts will meet with wide appreciation.—GEO. D. FULLER.

Flora Balearica.—In two large volumes KNOCHE[4] has given what appears to be a very complete catalogue of all the plants occurring on the Balearic Islands. The data have been accumulated both from the study of herbaria and from personal visits to the several islands. The notes on distribution are extensive and are supplemented, for the vascular plants, by sketch maps showing all the recorded localities of occurrence within the islands. The work abounds in details, but seems lacking in any generalized account of the

[3] CHASE, AGNES, First book of grasses: The structure of grasses explained for beginners. New York: Macmillan Co. 12mo. pp. xii+121. *figs. 94.* 1922.

[4] KNOCHE, HERMAN, Flora Balearica. Étude phytogéographique sur les Iles Baléares. Montpellier. 8vo. vols. 2. pp. 534 and 583. maps 2. 1921, 1922.

origin, affinities, or relationships of the flora. It should prove a useful mine of information for future students of the region.—GEO. D. FULLER.

Flora of Nebraska.—PETERSEN[5] has published a new edition of his *Flora of Nebraska*, which differs from the edition of 1912[6] chiefly in the inclusion of the ferns. Formal descriptions of species are omitted, but the enumeration of species is preceded by keys leading to their determination. This very convenient book has proved to be of much service to those who wish to become acquainted with the plants of Nebraska.—J. M. C.

NOTES FOR STUDENTS

Vegetation of South Australia.—The state of South Australia lies almost wholly within the desert and semi-arid regions of Australia, as only one-sixth of its area has a rainfall in excess of 10 inches per annum. In general the surface is relatively level, consisting of a series of plains and plateaus. CANNON[7] has recently made a considerable contribution to our knowledge of the vegetation of the region. His report contains a good synopsis of climatic conditions upon the continent, with maps, tables, and graphical comparisons of rainfall, evaporation, humidity, light, temperature, and winds. The physical geography of South Australia is also sketched, and studies of the vegetation of various areas given with a wealth of detail entirely impossible to reproduce. In fact, the main criticism to be made of an otherwise excellent report is that the many details tend to obscure the more important generalizations. This could have been remedied by the introduction of well organized summaries.

Three areas studied by CANNON may be taken as typically representative of the desert, the arid, and the semi-arid regions of Australia. In the Lake Eyre region the general aspect is very desert-like, with a rainfall of 5 inches or less, much of which comes in such light showers as to be inefficient in increasing soil moisture. Here upon the plains various species of *Eremophila* and a few annuals that spring up after the winter rains constitute the greater part of a very scanty vegetation. The species of *Eremophila* are mostly in the form of isolated, much branched shrubs, a meter high, with small dull green leaves. In the adjacent sand hills there is the addition of shrubby species of *Acacia*, sometimes reaching small tree size, and tufts of bunch grass. In the plains and low hills somewhat south of Lake Eyre, near Copley, with a rainfall of about 8 inches, the desert shrubs become larger, one of the peculiar features being considerable areas, each dominated by an open stand of one single shrub

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[5] PETERSEN, N. F., Flora of Nebraska. A list of ferns, conifers, and flowering plants of the state, with keys for their determination. 8vo. pp. 220. Published by the author, Plainview, Nebraska. 1923.

[6] BOT. GAZ. 56:80. 1913.

[7] CANNON, WM. A., Plant habits and habitats in the arid portions of South Australia. Carnegie Inst. Wash. Publ. 308: pp. vii+139. *figs.* 31. 1921.

species, such as *Eremophila freelingii*, *Philodia scoparia*, or *Cassia sturtii*, similar in their widely branching habit and in their height (1.5–2 m.).

Much of the western portion of South Australia may be termed arid rather than desert. This was visited by CANNON, and here too ADAMSON and OSBORN[8] have made an instructive study within an area with a little less than 10 inches of rain per annum, and on the transition from the limestone Nullarbor plain with shallow coarse soil to the sand hills to the east. The plain has a scanty covering of "bluebush," *Kochia sedifolia*, and "saltbush," *Atriplex vesicarium*, small gray bushes half a meter high, standing 2 to 3 m. apart, with a few annual herbs and a sparse scattering of grasses. This CANNON regards as essentially halophytic, an opinion with which the Australian investigators ADAMSON and OSBORN disagree. The latter also point out that the better conservation and utilization of the same rainfall in the sand hills results in an open forest. In the deepest sand there is a dominance of the "oak," *Casuarina lepidophloia*, which reaches a height of 50 feet and a trunk diameter of 26 inches. Smaller trees are *Myoporum platycarpum* and the "mallees." On the sand ridges the trees are reduced to shrubs, among which *Acacia* spp. and *Grevillea* spp. are notable.

An analysis of the flora according to Raunkiaer's biological spectrum shows the absence of large trees, the number of perennial herbs much below normal, but a marked excess of microphanerophytes and nanophanerophytes (small trees and shrubs) and of annuals. The more xerophytic areas show a close correspondence to Death Valley, California.

CANNON's studies of the Quorn region, with a rainfall of about 14 inches, may be taken as representing the semi-arid vegetation of shrubs and small trees, among which species of *Acacia*, *Casuarina*, *Eremophila*, *Cassia*, *Hakea*, *Olearia*, *Zygophyllum*, and *Eucalyptus* are abundant. In this region the "mallee scrub," one of the largest and most distinctive plant associations of Australia, is much in evidence. In the "mallee" habit the stem is shortened into a large bulbous base, which may be several feet in diameter, and according to MAIDEN functions in the storage of water. From this branches spring and attain the height of small trees, with the general aspect of large shrubs. The term "mallee" includes half a dozen or more species of *Eucalyptus*.

To the descriptions of vegetation CANNON has added notes on leaf form, leaf size, root features, phyllodia, and the reactions of various species to light, temperature, and small water supply. He has also given much information through a series of excellent plates from good photographs.

OSBORN[9] has also described the vegetation of some small islands south of Australia, where coastal dunes are dominated by *Spinifex* and *Olearia* shrubs,

[8] ADAMSON, R. S., and OSBORN, T. G. B., On the ecology of the Ooldea District. Trans. Roy. Soc. So. Australia **46**:539–563. *pls. 32–36.* 1922.

[9] OSBORN, T. G. B., Flora and fauna of Nuyt's Archipelago, no. 3. A sketch of the ecology of Franklin Islands. Trans. Roy. Soc. So. Australia **46**:194–206. *pls. 8–11.* 1922.

while the uplands are also occupied by shrubs of *Rhogodia crassifolia* and *Frankenia pauciflora*. Unstable soil and severe winds seem to account for this reduced vegetation.—GEO. D. FULLER.

Research methods in ecology.—Different stages in ecological investigation are recognized in some of the more recent publications dealing with methods of work. South Africa has undertaken a botanical survey of its relatively unknown area, and the director[10] has issued a pamphlet containing a sketch of the physical features of the country, a summary of its climatic conditions, a division into main botanical regions, a complete bibliography of its botanical literature, and in addition directions for collecting, surveying, and mapping the vegetation. Some useful hints on photographic records are also included.

BRAUN-BLANQUET[11] stresses the importance of closer analyses of plant communities regarded as social units. For this purpose he gives directions for a quantitative evaluation of the component species on the basis of such qualities as exclusiveness, constancy, abundance, frequency, dominance, and sociability.

Seeking for more accurate methods of estimating frequency, ARRHENIUS[12] examines those already in use, and decides that RAUNKIAER's is the best, although somewhat lacking in accuracy. He then describes one devised by himself, consisting of taking the plant population from a belt of suitable width alongside a rope marked in decimeters and meters. The vegetation is divided into two layers, an upper and a lower, and these are analyzed and charted separately. From these charts of the belt the frequency percentage and the number of individuals per unit area, or "absolute frequency degree," are easily calculated. This latter expression permits direct comparisons between various areas even when they have been studied by different individuals, and is one of the chief advantages of the method. Other advantages claimed are convenience, time saving, exact results from even a short belt, a simple expression of the closeness of the community, and a convenient means of analyzing zonation. The mass of vegetation is measured photometrically through the degree of shading.

These and other methods of phytosociological analysis are also adequately discussed by RÜBEL[13] in his exhaustive and excellent compendium of research methods. This is decidedly the best modern work on the subject, and is characterized by broad, judicious treatment, and by the attention given to habitat factors and their measurement. He figures and describes a large

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[10] POLE EVANS, I. B. et al., A guide to botanical survey work. Bot. Surv. So. Africa. Memoir 4: pp. 89. maps 5. 1922.

[11] BRAUN-BLANQUET, J., Prinzipien einer Systematik der Pflanzengesellschaften auf floristicher Grundlage. Jahrb. St. Gallischen Naturw. Gesells. **57**:305–351. 1921.

[12] ARRHENIUS, O., A new method for the analysis of plant communities. Jour. Ecology **10**:185–199. *fig.* 1. 1922.

[13] RÜBEL, EDWARD, Geobotanische Untersuchungsmethoden. Berlin. 8vo. pp. xii+290. *figs.* 70. 1922.

number of instruments, such as thermographs, photometers, and atmometers, showing himself familiar with their latest improvements. He has also some useful suggestions for cartography.

The emphasis now being placed upon the quantitative investigation of environmental conditions is well indicated by the simultaneous appearance in America of a publication on research methods[14] similar to that of RÜBEL'S, but limited in its scope to forest environment. Here, however, the treatment of soil moisture study is more adequate than that given by RÜBEL, and the bibliography is more conveniently arranged. There is, however, little in either work that deserves adverse criticism, and both will be indispensable to all interested in ecological research.—GEO. D. FULLER.

Assimilation of submerged plants.—A comprehensive study has been made by RUTTNER,[15] employing the conductivity method, of the course of absorption by submerged plants while growing in sea water, distilled water, or nutrient solutions, and exposed to light of various colors and to darkness. Particular attention is given to explaining the rhythmic changes in conductivity related to the sequence of day and night, and to the absorption of CO_3 and HCO_3 ions by the plant. Carefully washed shoots of *Elodea* were employed in all the experiments.

The inception of photosynthetic activity in daylight causes a fall in conductivity, due to the dissociation of bicarbonate and absorption of CO_2, while $CaCO_3$ is precipitated. In the presence of the plant this was found to occur at a much faster rate, and to proceed more nearly to completion than occurs in sea water or bicarbonate solution upon standing, or after long continued passage of CO_2 free air. *Elodea* was able to reduce the conductivity of a saturated bicarbonate solution to approximately $0.3 \times 10-4$ reciprocal ohms, which is that of a saturated solution of $CaCO_3$, and represents more complete removal of bicarbonate than can be produced by prolonged boiling. This occurred only in the presence of living *Elodea* exposed to light, and the conclusion is reached that submerged plants are able to utilize, in addition to dissolved CO_2, that combined in HCO_3 ion. WILMOTT,[16] employing the bubble method, concluded that water plants are incapable of decomposing bicarbonates, since the rate of dissociation of bicarbonate is nearly the same upon standing as in the presence of vegetation; but RUTTNER'S method is by far the more sensitive. The

[14] BATES, C. G., and ZON, RAPHAEL, Research methods in the study of forest environment. U.S. Dept. Agric. Bull. 1059. pp. 208. *figs.* 4. 1922.

[15] RUTTNER, FRANZ, Das electrolytische Leitvermögen verdünnter Lösungen unter dem Einflusse submerser Gewächse. Sitzungsber. Akad. Wiss. Wien **130**[1]:71–108. *figs.* 4. 1921.

[16] WILMOTT, A. J., Experimental researches on vegetable assimilation and respiration. XIV. Assimilation by submerged plants in dilute solutions of bicarbonates and acids: an improved bubble-counting technique. Proc. Roy. Soc. London B **92**:304–327. 1921.

latter attributes the power of plants practically to exhaust solutions of their HCO_3 ions to the absorption of CO_3 at a more rapid rate than Ca, with resultant increase in concentration of OH ions. The same process accounts for the rise in conductivity and production of an alkaline reaction under intense assimilation. The reverse change causes a decline in conductivity with diminishing photosynthetic activity; a second rise, due to respiratory activity, occurs during the night.

Based upon the amount of CO_2 precipitated and the change in conductivity, the rate of assimilation was determined. In brook water this was found to be 8 mg. of CO_2 per gram of dry substance per hour, while 18.1 mg. of $CaCO_3$ was precipitated. A colony of *Elodea* having a green weight of 100 kg. may deposit 2 kg. of lime each day of 10 hours of sunlight. To this process is due in considerable measure the deposition of chalk in shallow sea water and along the shores of rivers.—FREEMAN WEISS.

Hydrogen ion and growth.—The influence of hydrogen ion concentration on the growth of *Giberella Saubinetii* has been investigated by HOPKINS,[17] who finds that there is a wide range of tolerance on the part of the organism. With several different methods of adjusting acidity, however, he has found that there is a growth minimum at about P_H 5.5, and this same degree of acidity in the soil also shows a minimum seedling infection by the parasite. Not only is the fungus affected, but the host plant also shows a rate minimum for germination at about this concentration. He suggests partial control of the scab infection by adjustment of the soil to this level of acidity. HOPKINS does not offer an explanation of the growth minima observed in these organisms toward the middle region of the tolerance range. If it were found to hold for many kinds of organisms, it might indicate a reduced activity of the living colloids at approximately the isoelectric point. In such colloids as albumen, casein, and gelatin, there is a depression of osmotic pressure, viscosity, and swelling at the isoelectric point which occurs at P_H 4.7–5.2 in these colloids. LOEB[18] considers that the proteins at the isoelectric point are unionized, and non-reactive. On either side of the isoelectric point they become reactive, on the more acid side as bases, on the less acid side as acids. He represents the nonreactive, acid reactive, and base reactive conditions by the following formulas respectively:

$$\left[R\begin{matrix}-NH_2\\-COOH\end{matrix}\right],\quad \left[R\begin{matrix}-NH_2\\-COOH,\end{matrix}\right]\quad \text{and}\quad \left[R\begin{matrix}-NH_2\\-COOH\end{matrix}\right].$$

The isoelectric point for living proteins is not very accurately known, and may vary with different organisms. They may not react in the same way as gelatin, casein, or albumen. Nevertheless, the depression of growth curves

[17] HOPKINS, E. F., Hydrogen ion concentration in its relation to wheat scab. Amer. Jour. Bot. **9**:159–179. 1922.

[18] LOEB, J., Proteins and the theory of colloidal behavior. New York: McGraw-Hill Co. 1922.

near the isoelectric point of other proteins suggests the possibility that the normal reactivity of the living proteins may be interfered with at hydrogen ion concentrations which retard ionization. Suppression of normal reactivity might thus retard the growth rate at such critical hydrogen ion concentrations. Whether this is true could be determined only by appropriate investigation.—C. A. SHULL.

Montana forests.—In a well written and attractively illustrated volume, KIRKWOOD[19] has discussed the forests of Montana. The scope of the work includes a historical sketch of the botanical exploration of the region, a consideration of the topographic and climatic features, an examination of the evidence of the origin of the flora from that of the Tertiary age or from those of adjacent regions, and an account of the aspect, zonation, and development of existing forests. In it the author has discussed the forest vegetation of Montana and a part of adjacent Idaho, an area of 175,000 square miles west of the 104th meridian and between the parallels of 45° and 49° north latitude. It is a mountainous region, the altitude ranging from 1800 to 10,000 feet, with the continental divide passing through it from north to south, about 100 miles east of the western boundary of Montana.

The rainfall ranges from 10 to 24 inches, or more in small local areas, the western slope being the more adequately watered. Nearly one-half of the area is or has been forested. While there is little evidence of any connection with a Tertiary flora, several deciduous tree species, such as *Populus* spp., *Prunus americana*, *Acer Negundo*, and *Fraxinus lanceolata*, appear to have come from the east, and a majority of the 17 species of conifers to have entered the area from the west or northwest. The deciduous species are largely confined to the bottom lands and stream banks, while the conifers dominate the remaining more exposed areas. Important species throughout the entire region are *Pinus ponderosa*, *P. contorta* (*Murrayana*), *Picea Engelmannii*, and *Juniperus scopulorum*, while west of the divide *Larix occidentalis*, *Pinus albicaulis*, *P. monticola*, *Tsuga heterophylla*, *Abies grandis*, and *Thuja plicata* are abundant locally. The percentage composition is given for 15 sectional areas into which the whole region is divided, and these data are among the most valuable contained in the volume.

The life zones of MERRIAM are but indistinctly represented, and nowhere clearly defined. Notes are given on the ecology of the various forest types, and on the leading species. A bibliography of 75 titles is appended.—GEO. D. FULLER.

Frost resistance and pentosan colloids.—A contribution to our knowledge of the physiological aspects of cold resistance has been made by ROSA,[20] who

[19] KIRKWOOD, J. E., Forest distribution in the northern Rocky Mountains. Univ. Mont. Studies no. 2. Bull. 247. pp. 180. *figs. 45.* 1922.

[20] ROSA, J. T., JR., Investigations on the hardening process in vegetable plants. Mo. Agric. Exp. Sta. Research Bull. 48. 1921.

has studied the process of hardening in such plants as cabbage, lettuce, and celery, where frost resistance varies with the growth conditions. Following the work of HOOKER,[21] who had pointed out that the more cold resistant parts of apple shoots had a greater water retaining power, and a correspondingly larger pentosan content, ROSA has shown that there is a rapid increase in the percentage of hydrophilous pentosans in these vegetables during the hardening process. On the other hand, plants like the tomato and sweet potato, which cannot be made frost resistant by any known treatment, show no significant increase in water retaining power or in pentosan content, when exposed to the same conditions that produce frost resistance in the cabbage. There is thus an important correlation between frost resistance, water retention, and pentosan content. The methods of hardening plants are discussed, complete analyses of the carbohydrates are presented, and there is a well ordered and excellent review of the extensive literature on this topic.—J. W. BUSHNELL.

Mixed plant communities in time.—The well known phenomena of various seasonal successions upon the same area is so much accentuated in the monsoon region of India, that SAXTON[22] regards as a new concept the fact that very different plant communities appear during the wet and dry periods. He points out that the same region may at one time of the year be a xerophytic bushland and at another a marsh. Thus two (or even three) different units of vegetation may regularly alternate with one another, giving rise to what he terms "mixed formations in time." His use of the term "formation" for such semi-independent units of vegetation seems unfortunate, but it is quite probable that the extremes of the monsoon climate justify some such emphasis on seasonal change of aspect as he indicates. He suggests that Indian ecological conditions differ so widely from those of Europe and America that it is useless to try to fit the results of the one land into the systems of the others. While this may be true, a careful study will surely show that the same principles must apply, however different the details.—GEO. D. FULLER.

Slugs and fungi.—BULLER[23] has been conducting some interesting chemotactic experiments, to test "the supposition that slugs find their food, and in particular find fungi, by their sense of smell." He describes seven experiments with *Phallus impudicus*, *Russula heterophylla*, and *R. nigricans*, in relation to the slug *Limax maximus*. He found that these fungi, under certain conditions in the open, attract the slug from a distance of at least 10–21 feet. Considering the shortsightedness of slugs, and the fact that they find their food at night, he

[21] HOOKER, H. D., JR., Seasonal changes in the composition of apple spurs. Mo. Agric. Exp. Sta. Research Bull. 40. 1920.

[22] SAXTON, W. T., Mixed formations in time: A new concept in oecology. Jour. Ind. Bot. 3:30–33. 1922.

[23] BULLER, A. H. R., Slugs as mycophagists. Trans. British Mycol. Soc. 7:270–283. 1922.

concludes that they react to odors given off by fleshy fungi. That they are remarkably sensitive to odors he demonstrated by their reaction to mustard gas when diluted to one part in ten million. He thinks that if the chemotaxis of slugs were understood, "we might perhaps be able to devise much more efficient means for protecting our gardens from the ravages of slugs than any at present known."—J. M. C.

Flora of Santa Catalina.—MILLSPAUGH and NUTTALL[24] have made an important contribution in publishing a very full account of the flora of Santa Catalina Island, since the natural conditions are being changed rapidly by "the advancing activities of man." The special interest of the flora is its large number of endemic species, greater than any equal area of the Californian mainland. To explain the origin of these endemic species involves the geologic status of the island, and a record of the flora is essential to the explanation. An account of the topography of the island is followed by a description of the principal collecting stations, 54 in number, and also a list of collectors and bibliography. A summary of the flora shows 455 spermatophytes, 12 pteridophytes, 36 bryophytes, 213 fungi, and 166 lichens, including descriptions of 47 new species. Gymnosperms and algae are not represented.—J. M. C.

Growth in trees.—In order to facilitate studies of the rate of growth in the diameter of tree trunks, MACDOUGAL[25] has invented a dendrograph which makes a continuous record of variations of tree trunks. A simpler instrument, known as a dendrometer, is also described, by which the size of the trunk may be read from time to time on a dial. Dendrographic records of trees of several different species are also presented. As a result of such records, it is apparent that enlargement of tree trunks is limited to a comparatively brief period of time, the awakening of the terminal buds often beginning some time before any increase in trunk diameter occurs. All tree trunks show a daily variation due to temperature and water content differences. The influence of the latter was apparent very soon after the addition of soil moisture by irrigation.—GEO. D. FULLER.

Embryogeny of Labiatae.—SOUÈGES,[26] in continuation of his extensive studies of embryogeny, has published the results of investigations of the Labiatae, the species reported on being *Mentha viridis*, *Glechoma hederacea*, and *Lamium purpureum*. The descriptions are full and abundantly illustrated.—J. M. C.

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[24] MILLSPAUGH, C. F., and NUTTALL, L. W., Flora of Santa Catalina Island. Field Museum Nat. Hist. Publ. 212. pp. 413. *pls. 14*. January 1923.

[25] MACDOUGAL, D. T., Growth in trees. Carnegie Inst. Wash. Publ. 307. 8vo. pp. 41. 1921.

[26] SOUÈGES, R., Recherches sur l'embryogénie des Labiées. Bull. Soc. Bot. France 68:441–464. *figs. 100*. 1922.

WORLD-ROMIC SYSTEM

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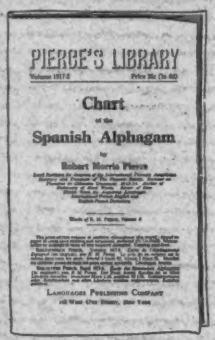

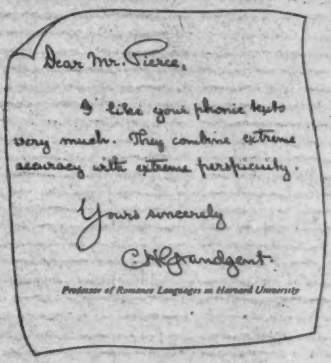
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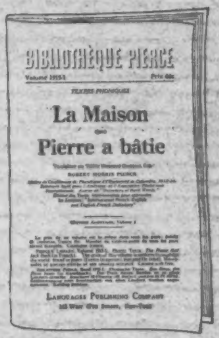

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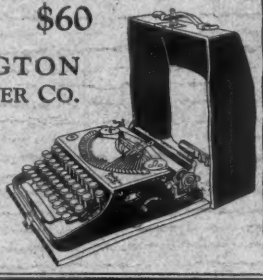

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